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NOTES ON TWO VENEZUELAN XIPHIDIOCERCARIAE*

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About five hundred specimens of *Ampullaria crassa Spix*, the only species of fresh water snail found in a small brook in the outskirts of Valera, Venezuela, were examined during January and February, 1924. Two methods were used for examination of this material. One was to crush the shell of the snail, remove the tissues and examine them under low power after being teased in 0.4 per cent. saline solution. The other method was to put several specimens of the snail in jars with a small amount of water, expose them to the sun for two hours and then examine the sediment under low magnification. In every case when the snails were infested with mature cercariae they showed under such conditions motile forms in the sediment. By dividing and subdividing the lots of snails, the infected ones were isolated, and thus furnished a supply of living material available for further study.

Cercariae were studied alive, and under favorable conditions most of the anatomy may be made out in the living organism. On account of the change of shape and the rapid movement of the cercariae, only material preserved in 5 per cent. formalin was used for measurements. The organisms evidently contracted to some extent in the course of preservation so that the measurements are slightly less than those of the living material. Tissue from parasitized snails was fixed in Bouin's picroformol, imbedded in paraffin, sectioned at five microns, and stained with dilute Delafield's hematoxylin. Cercariae for mounting in toto were fixed in warm Bouin's fluid, stained with very dilute Delafield's hematoxylin and differentiated in 95 per cent. alcohol acidulated with hydrochloric acid. Canada Balsam was used for mounting.

Out of about five hundred snails examined, two species of stilet cercariae were found which will be described in this paper.

CERCARIA REPTANS n.sp.

In 5 per cent. of the *Ampullaria crassa* collected, the liver was found to be extensively invaded with rather small sporocysts which were easily

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isolated from the liver tissue. In some cases the infestation was so heavy that the liver was almost replaced by a mass of sporocysts. The sporocysts are sac-shaped, measuring from 0.6 to 1.25 mm. in length and from 0.3 to 0.4 mm. in width. They are motionless except for slight movement due to the slow wriggling of the cercariae which they contain. At one extremity a knob-like structure is seen which seems to be the point of attachment of the sporocyst to the liver tissue. In the other extremity is the germinating epithelium but few germ-balls are seen. The sporocysts when mature have from two to five cercariae which very readily escape through the thin wall. The name *Cercaria reptans,* is proposed for this species on account of its creeping locomotion.

Cercaria reptans (Fig. 1) has an oval shaped body with the anterior extremity slightly compressed, and its posterior portion tapering from the region of the acetabulum. The cross section is somewhat oval but flattened on the ventral side. In the living cercaria the fully extended tail is much longer than the body, but is capable of contracting to about two-thirds its length when extended. When contracted it shows marked transverse folding of the cuticula (Fig. 6). The average length of body in cercariae preserved in 5 per cent. formalin is 0.26 mm., the average width 0.13 mm. The measurements of the tail vary much in such material according to the degree of extension or contraction at the time of fixation. After measuring a series of individuals it was found that it varies from 0.32 to 0.2 mm. in length by 0.03 to 0.032 mm. in width at its base.

Cercaria reptans is a very poor swimmer, but by the alternate use of its suckers, creeps along very rapidly somewhat in the fashion of a measuring-worm. As it creeps the tail shows a peculiar pulsation which does not seem to assist in locomotion. When the cercaria becomes attached to any kind of foreign material, the body twists itself around, while the tail lashes violently until the organism is set free and the creeping is resumed. Tailless cercariae were observed creeping as readily as those with tails. Detached tails have a slow movement of bending and stretching without changing place in any definite direction.

The oral sucker of *Cercaria reptans* is located in the ventral aspect of the anterior extremity and has an average diameter of 0.062 mm. The acetabulum is located at about two thirds of the distance from the anterior extremity. It is subspherical in shape and has an average diameter of 0.065 mm. A very conspicuous stilet is set in the dorsal wall of the oral sucker. It is a sharply pointed rod and possesses a thickening on its ventral side, one-third the distance from the tip to the base. The dorsal side is smooth and slightly bent (Figs. 2, 3). The stilet has a length of 32μ and a width at its base of 5μ. The middle third of the dorsal surface of *Cercaria reptans* is darkened by a delicate

brown pigment which makes the observation of the inside structures very difficult. The whole surface of the body is thickly covered with fine cuticular spines arranged in the usual diamond pattern. The caudal pocket which is situated somewhat ventrally at the posterior end is bordered by a thickening of the cuticula. On both sides are seen the locomotor grooves (Figs. 1, 9) set with thick spines.

The ventrally situated oral aperture is followed by a small mouth cavity and prepharynx. A small pharynx is located at the beginning of the esophagus, which is short and narrow. The intestinal crura do not reach to the posterior portion of the body. Both esophagus and intestinal crura are lined by a thin epithelial layer. The intestinal crura are covered externally with small glands which are clearly seen only in sections (Figs. 5, 11, 12). These glands are similar to the ones described by Faust (1917) for the pharynx, esophagus and intestine of *Cercaria glandulosa*. The glands of the salivary mucin type, i. e., the stilet glands, are arranged in two clusters of eight or more cells each, which extend posteriorly to the sides of the acetabulum (Fig. 1). These glands possess a very large nucleus which does not stain readily with hematoxylin. Two strands of gland ducts arise from the cell clusters and after making a sharp curve on the sides of the oral sucker (Figs. 1, 7), open at the dorsal side of the stilet (Fig. 17). The excretory vesicle is of the bicornuate type. The tubular portion is very muscular, subcylindrical in shape and deeply crenated. From this rise two short lateral cornua which embrace the posterior portion of the acetabulum. The main excretory tubes extend anteriorly from the tips of the cornua to the vicinity of the oral sucker. The inner surface of the excretory vesicle and lateral cornua is lined with a thin epithelial layer (Fig. 16). Around the body of the excretory vesicle, there are large muscle cells that are clearly seen in fresh material in which they mask the true shape of the vesicle. They are brown in color and possess a large nucleus which in sections shows rather sparse peripheral chromatin and contains two small excentric chromatic granules unequal in size (Figs. 15, 16).

The genital system of *Cercaria reptans* cannot be distinguished in unstained material. By using gross mounts stained with dilute hematoxylin, the genital system was found to be indicated by a collection of small deeply stained nuclei situated at one side of and immediately behind the acetabulum (Figs. 7, 8, 14). The genital pore is situated immediately anterior to the acetabulum. The uterus, ovary, and testis are not at all differentiated. A large accumulation of nuclei situated at one side of the anterior border of the acetabulum, probably represents the future uterus. The vitellaria are distributed diffusely along the sides of the body, extending from the region of the oral sucker to the postacetabular region.

Encystment was not observed *in vitro*. Attempts to infect tadpoles were positive. Encysted stages were found one hour after feeding infected liver tissue to tadpoles. Cysts were found in small numbers in the intestine and very numerous in the soft tissues surrounding the mouth cavity of the tadpole. The cysts are round or more or less oval in shape. When oval they have a longitudinal diameter from 0.18 to 0.218 mm., and a transverse one from 0.16 to 0.17 mm. The wall is very thin and transparent. The coiled agamodistomum, which moves rather actively, is clearly seen and most of its structures sharply outlined.

Except for the comparative size of the suckers which are in some instances equal in size but usually with acetabulum slightly larger than oral sucker, and except also for the shortness of the intestinal crura which do not reach to the posterior end of the body, *Cercaria reptans* coincides very closely with the cercariae classified in the artificial group of polyadenous cercariae which are distinguished by the following characteristics given by Cort (1915):

1. Development in gastropods in elongate, sac-shaped sporocysts.
2. Tail slender and shorter than the body except when very much extended.
3. Acetabulum posterior to the middle of the body and smaller than the oral sucker.
4. Stilet about 0.03 mm. in length, six times as long as broad, and with a thickening one third of the distance from the point to the base.
5. Stilet glands six or more on each side, between the acetabulum and the pharynx.
6. Excretory bladder bicornuate.
7. Very short prepharynx and small pharynx present. Oesophagus when developed of short to medium length. Intestinal caeca, when present, reach to posterior end of body.

Cercaria reptans seems to belong to a subgroup of the polyadenous cercariae, which subgroup would be characterized by the presence of numerous glands around portions of, or around the entire digestive tract. *Cercaria glandulosa* Faust (1917) would be the type of this subgroup. *Cercaria glandulosa* and *Cercaria reptans* are easily distinguished by the difference in size of body and by the size and ratio of suckers and by the presence of glands around the pharynx and esophagus in *Cercaria glandulosa*. *Cercaria isocotylea* Cort (1915) has suckers of about the same size, but differs by being much smaller in size and by the absence of locomotor grooves which are conspicuous in *Cercaria reptans*.

Suggestions have been made in regard to the type of adults into which the polyadenous cercariae develop. *Cercaria limneae ovatae*

Lühe (1909) and *Cercaria secunda* Ssinitizin (1905), two fresh water cercariae from Europe, have been assigned to *Opisthioglyphe rastellus* and *Plagiorchis* sp., respectively. No statement can be made regarding *Cercaria reptans* but since it is closely related to the polyadenous cercariae and its anatomy is so similar to some of the adults of the family Plagiochiidae Lühe it is probably at least closely related to this family.

ENCYSTMENT OF CERCARIA REPTANS

About one hundred and twenty tadpoles were secured from a small pond where no snails have been found to occur. Seventy of the younger of these were used for the feeding experiments and the rest kept as controls in a separate jar. Some *Batracospermun* sp. and *Chara* sp. weeds were put in the jars in order to keep the water freed from mosquito larvae. The seventy tadpoles selected were fed the liver of a heavily infected snail, which was eaten by the tadpoles in a very short time. Twenty minutes later three tadpoles were killed, their tissues teased in salt solution and examined at a low magnification. A few cysts were found already in the soft tissues around the mouth cavity and the tissues surrounding the eyes. Three control tadpoles were examined and found free from any kind of encysted cercaria.

Thirty tadpoles of the lot thus infected were put into a separate jar with a small amount of water. The livers from three heavily infected snails were teased into small bits and placed in the water with these tadpoles. The number of cercariae freed was enormous. After the cercariae were put in the water, it was observed that the tadpoles became unusually restless, swimming very fast and coming very often to the surface of the aquarium. Fifteen minutes later three tadpoles were examined. An enormous number of cysts were found in the tissues surrounding the natural cavities and the eyes. Several cercariae were found still alive in the lumen of the intestine and a few cysts were seen in the intestinal wall. Two of the three tadpoles examined showed a few cysts under the skin of the transparent membrane of the tail. They were seen in the living tapoles and seemed to be just under the skin.

Two more livers from infected snails were now placed in the jar containing the twenty-seven tadpoles which remained. Immediately after the cercariae were freed in the water the same excitement previously noted was observed. Twenty-four hours after this second feeding ten tadpoles were found dead. They showed extensive invasion of all the tissues examined. Of the rest of this lot four were killed and fixed in warm Bouin's fluid together with five of the ones found dead. The forty tadpoles which had been fed on one occasion only with infected liver tissue remained alive. For five subsequent days small pieces of infected snails liver was put in the jar. In this way we obtained a very

heavy infestation but only two died during this time. Every second day one or two were killed to follow the development of the encysted worm but, outside of the granules which accumulated in the excretory vesicle, no appreciable change occurred during one month. The tapoles fixed in Bouin's fluid were imbedded in paraffin and sectioned at ten microns. Some sections were stained with hematoxylin and eosin and others by the Giemsa method. Sections of the cysts were clearly detected in almost every tissue. Cysts are found under the skin (Figs. 1, 4), around the eyes (Fig. 3), in the muscles (Figs. 1, 2, 6), inside the lateral ventricles (Fig. 4), and in the brain substance itself (Fig. 5).

Beyond a mild cellular infiltration surrounding the encysted cercariae, no important pathological reaction was apparent. The sudden infection with great numbers of cercariae which invaded vital places, may account for the death of the tadpoles exposed to such enormous numbers. The ones which were infected gradually supported infections quite as heavy as the ones which died. As can be seen by experiment most of the Xiphidiocercariae enter the host through the natural cavities as Lutz has pointed out. However, many of them doubtless pierce the skin and the soft membranes around the eye, using the stilet for that purpose.

CERCARIA FAUSTI

The liver of 2 per cent. of the 500 specimens of *Ampullaria crassa* sp. collected were heavily parasitized with colorless, oval-shaped sporocysts measuring from 0.3 to 0.5 mm. in length by 0.1 to 0.2 mm. in width. The sporocysts seemed to be attached to the liver tissue by a small knob-like structure projecting from one extremity. On the opposite end the germinating epithelium is found. The sporocysts have a transparent wall through which numerous germ balls and cercariae in different stages of development are clearly seen. The mature cercariae move actively inside the sporocyst which is motionless except for the slow movement given to it by the wriggling of the cercariae. The cercariae escape from the sporocyst by means of their anterior extremity with which they piece the thin limiting membrane. This rocess was observed on several occasions. The sporocyst as it appears while the process of piercing is going on is illustrated diagramatically in Figure 27. The name of *Cercaria fausti* is proposed for this cercaria in honor of Dr. Ernest Carroll Faust.

Cercaria fausti (Fig. 20) is a small pear-shaped cercaria. Its body is more or less pointed at its anterior extremity and rounded posteriorly with a well marked caudal pocket, located slightly ventrally. It is nearly twice as long as broad and in cross section is oval. In living specimens the tail is longer than the body but it can be retracted to less than the body length. In material preserved in 5 per cent. formalin, the

body measures from 8 to 95μ in length by 55 to 65μ in width. The tail is from 70 to 115μ long by 18μ wide. The posterior extremity of the tail is suddenly constricted into a short blunt point.

This cercaria moves very actively when freed from the sporocyst. The tail lashes actively to the sides, while the anterior extremity stretches out and retracts itself into some sort of a cephalic pocket which is clearly seen when the cercaria retracts the oral sucker (Figs. 23, 24). By the alternate stretching and retracting of its anterior extremity, together with the active lashing of its tail, *Cercaria fausti* shows a type of movement in some respects similar to the one described for *Cercaria reptans,* but it does not creep like the latter does, due to the fact that the suckers are not yet fully developed and are not functional. In all the specimens of *Cercaria fausti* observed, the suckers were incompletely developed. The oral sucker is represented by a terminal mass of cells, which take the shape of the anterior extremity when it is stretched out. When the cercaria is at rest it is ovoid in shape and well demarcated from the rest of the body. Its longitudinal diameter is 27μ and the transverse 25μ. Set in the dorsal wall of the oral sucker is the stilet (Fig. 21), a sharply pointed rod measuring 21μ in length. It is reinforced along its length and possesses a thickening at one third of the distance from the tip to the base. This thickening seems to be confined to the ventral side and appears to be crenated. The acetabulum is situated back of the middle of the body and is very indistinctly outlined. In a very few cases, in which an approximate measurement was made, it was found to have a diameter of 16 to 17μ. The whole surface of the body and tail of *Cercaria fausti* is thickly covered with very fine cuticular spines which are thicker on the anterior extremity and on the tip of the tail. The caudal pocket is situated at the posterior extremity of the body and shows no locomotor grooves. Small cells which seem to be glandular in character are situated on both sides of the caudal pocket (Fig. 20).

The digestive system is represented by an undeveloped mouth cavity and a small muscular pharynx. The esophagus and intestinal crura are not at all defined. On the sides of the acetabulum, two clusters of voluminous cells of the salivary-mucin type or stilet glands are seen. It is rather difficult to ascertain the number of these glands, but they seem to be four on each side. From the glands start the gland ducts which are very thick and filled with coarse granules; they open on both sides of the stilet. The excretory vesicle is bicornuate (Figs. 20, 26), having a very small body and two large cornua which do not extend anteriorly farther than the posterior border of the acetabulum. At the tips of the cornua, and when the vesicle is distended, two small round dilatations are often seen. The excretory pore is situated slightly dorsal to the posterior extremity.

The genital system, which does not show any differentiation, is represented by a mass of cells just behind the acetabulum. It shows rather marked lobes; two anterior and one posterior (Fig. 25). The vitellaria cannot be made out. No encystment of *Cercaria fausti* was observed. Infected liver tissue was fed to tadpoles but the numerous cercariae which were found in the intestine did not live more than a few hours. After twenty-four hours several tadpoles were examined, and no trace of the cercariae was seen.

Cercaria fausti is somewhat similar in its morphology to *Cercaria leptacantha* (Cort, 1915), but differs from it in the size and shape of the stilet and by the possession of a caudal pocket. It seems to belong to a provisional group of very small cercariae called Cercariae microcotylae (Lühe, 1909). They are distinguished by the following characteristics given by Cort (1915):

1. Develop in gastropods into round or oval sporocysts which are seldom more than twice as long as wide.

2. Cercariae under 0.2 mm. in length.

3. Acetabulum back of the middle of the body and smaller than the oral sucker.

4. Stilet glands not more than four on each side and arranged in rows on each side of the acetabulum.

5. Digestive system undeveloped except for a short prepharynx and a small pharynx.

Looss (1896) suggests that the three Xiphidiocercariae of this type described by him may be related to some small Distomes found in Egypt in the intestine of chameleons and lizards. No suggestion can be made at present as to what type of adult worm *Cercaria fausti* develops into.

SUMMARY

Two Xiphidiocercariae are described from the liver of *Ampullaria crassa* Spix collected in Valera, Venezuela, S. A. Comparison with cercariae already described shows that they are two new species.

Cercaria reptans belongs to the group of Polyadenous cercariae, under a subgroup characterized by the presence of glands around part of, or the entire digestive tract, *Cercaria glandulosa* Faust (1917) being the type of this subgroup.

Cercaria fausti is closely related to the group Cercariae microcotylae Lühe (1909).

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Abbreviations Used

ac Acetabulum	*nc* Nervous commissure
ao Cartilage	*lc* Laurer's canal
c Cysts	*lg* Locomotor grooves
cg Caudal glands	*oe* Esophagus
co Excretory cornua	*om* Tail membrane
cp Caudal pocket	*os* Oral sucker
eo Blood vessel	*p* Pigment
ev Excretory vesicle	*ph* Pharynx
g Caudal pocket glands	*pr* Prepharynx
gc Gland ducts	*pt* Posterior tube
gp Genital pore	*sg* Stilet glands
gs Genital system	*cst* Excretory tube
ic Intestinal crura	*sti* Stilet
lg Intestinal glands	*v* Ventricle
vm Vesicular muscle cells	

EXPLANATION OF PLATE XVIII

(Magnifications indicated are approximate)

Figures 1 to 19 represent *Cercaria reptans*

1.—Ventral view; stilet glands shown only on left; intestinal glands on right. × 200.

2.—Ventral view of stilet. × 500.

3.—Lateral view of stilet. × 500.

4.—Mouth cavity and prepharynx (diagrammatic). × 200.

5.—Intestinal glands (diagrammatic). × 200.

6.—Diagram of retracted tail showing transverse folding of cuticula. × 200.

7.—Lateral view showing position of genital system. × 200.

8.—Genital system. Hematoxylin stain. × 200.

9.—Posterior extremity showing excretory vesicle, vesicular muscle cells and locomotor grooves. × 200.

10.—Encysted in tissues of tadpole. × 150.

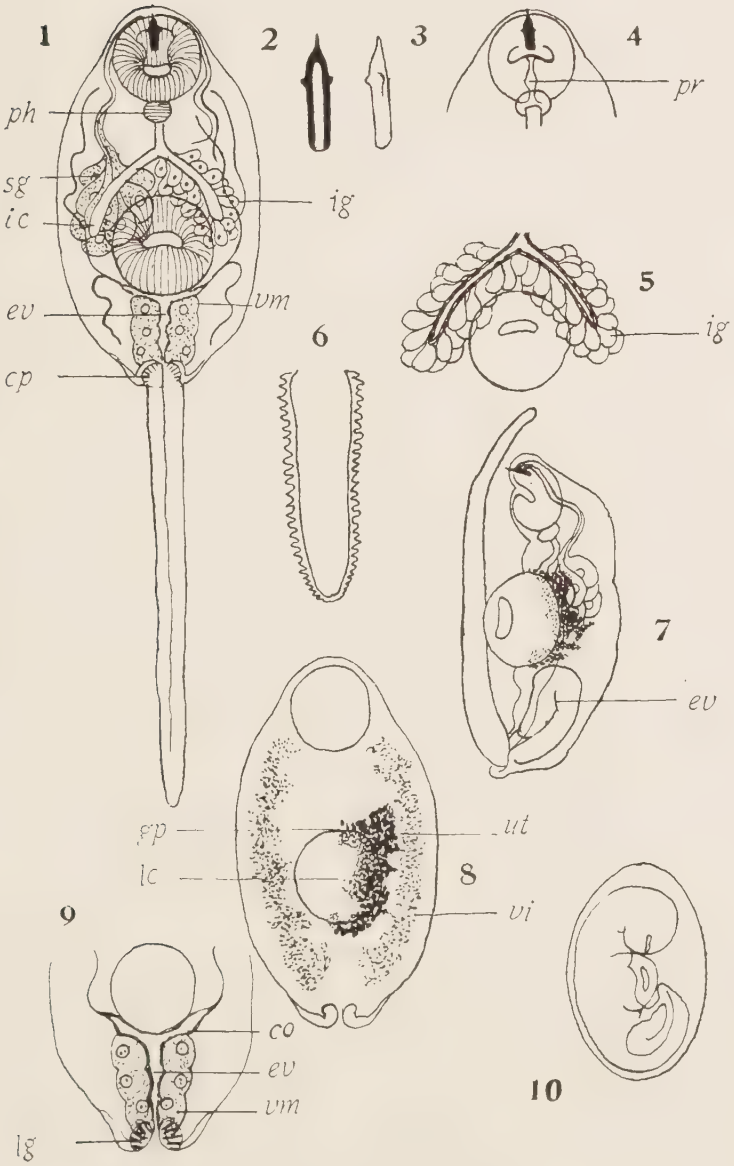

PLATE XVIII

Explanation of Plate XIX

11.—Front section showing distribution of intestinal glands. × 250.

12.—Frontal section showing nervous commissure. × 200.

13.—Diagonal section showing prepharynx, pharynx and esophagus. Two stilet glands are seen. × 250.

14.—Longitudinal section showing genital system. × 250.

15.—Longitudinal section showing vesicular muscle cells. × 250.

16.—Longitudinal section of posterior extremity showing excretory vesicle, cornua, excretory tube and vesicular muscle cells. × 300.

17.—Diagonal section in region of acetabulum, showing stilet glands and gland ducts. × 250.

18.—Section of posterior extremity showing vesicular muscle cells surrounding excretory vesicle. × 250.

19.—Transverse section of tail showing caudal glands and longitudinal excretory tube. × 1,000.

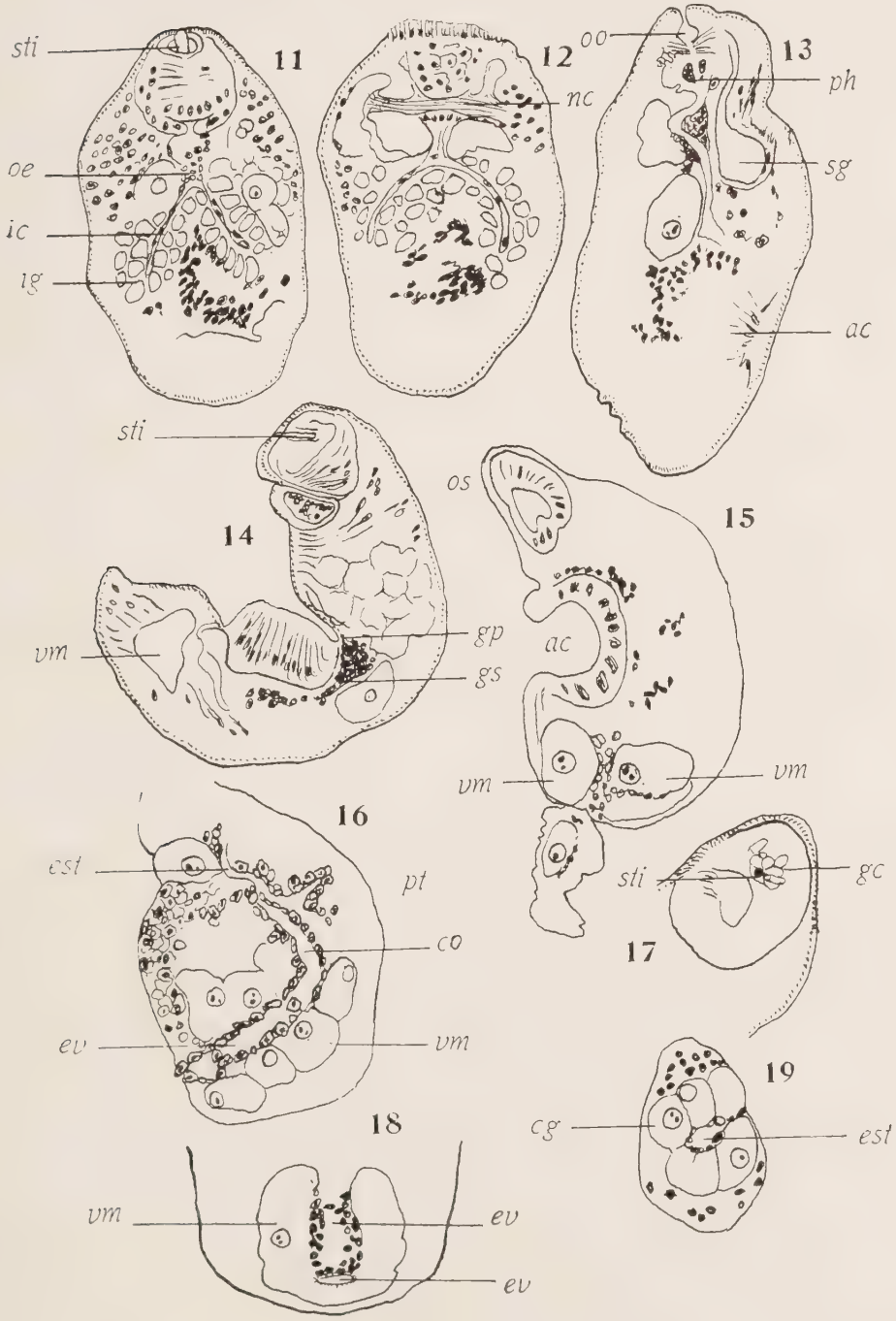
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PLATE XIX

Explanation of Plate XX

Figures 20 to 32 represent *Cercaria fausti.*

20.—Ventral view. × 500.

21.—Ventral view of stilet. × 1,000.

22.—Lateral view of anterior extremity. × 500.

23, 24.—Diagrams showing retraction of anterior extremity into cephalic pocket. × 250.

25.—Diagram of genital system. × 1,000.

26.—Excretory vesicle. × 1,500.

27.—Sporocyst showing cercaria pushing out wall. × 200.

28.—Different aspects. × 120.

29.—Transverse section at level of anterior border of acetabulum. × 500.

30.—Transverse section at level of acetabulum. × 500.

31.—Transverse section of oral sucker showing stilet and gland ducts. × 500.

32.—Diagonal section of posterior extremity showing cells around caudal pocket. × 500.

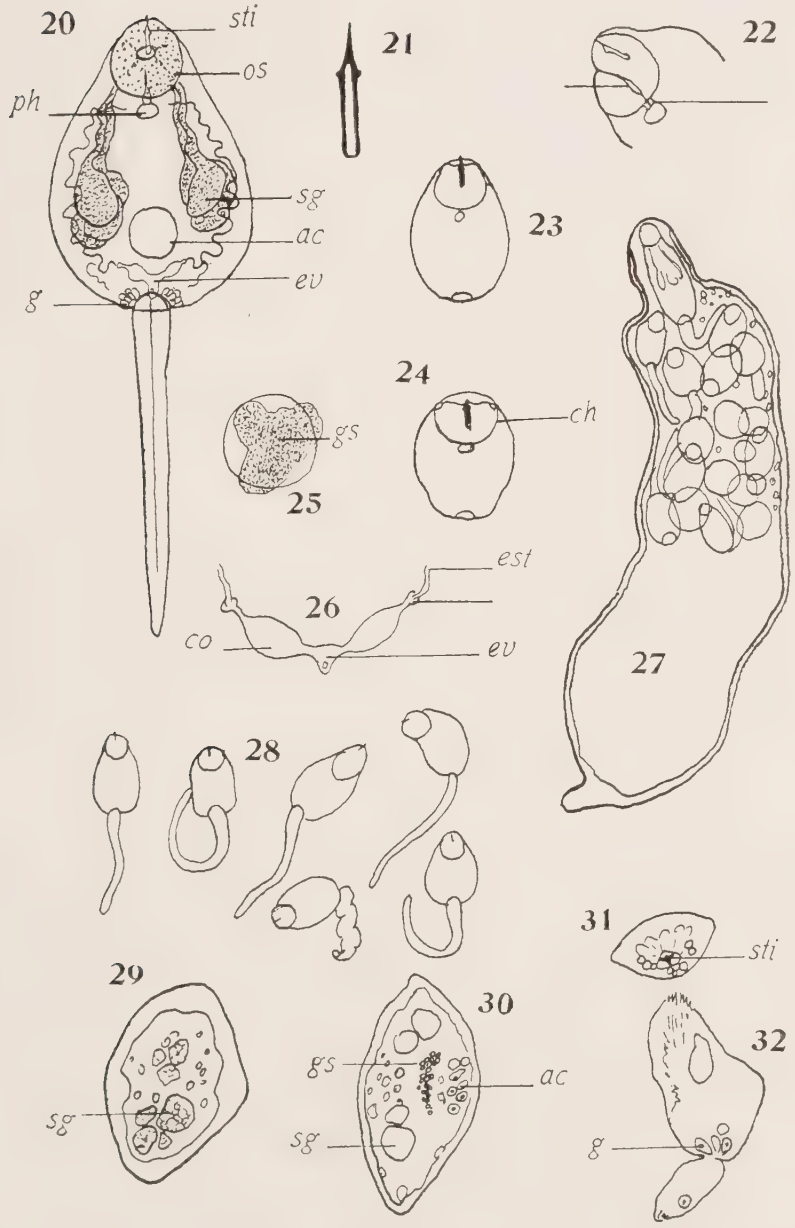
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PLATE XX

Explanation of Plate XXI

Description of Plate

Cercaria reptans

33.—Encysted in superficial tissues of head of tadpole.

34.—Encysted under the skin of tail of tadpole.

35.—Encysted under conjunctiva in orbit of tadpole.

36.—Encysted outside and inside meninges and in ventriculus of brain of tadpole.

37, 38.—Sections showing cercariae encysted in different portions of brain and medulla.

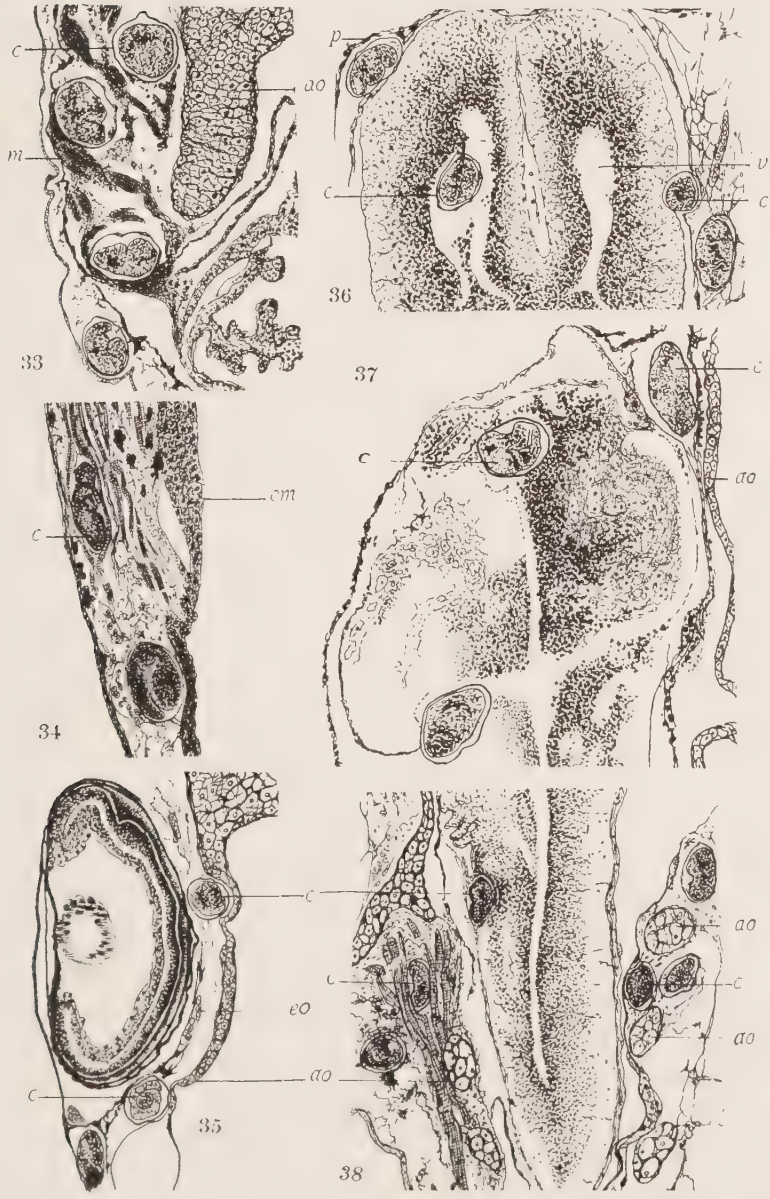

PLATE XXI

NOTES ON *ZYGOCYSTIS COMETA* STEIN, A GREGARINE PARASITE OF EARTHWORMS*

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The following notes on *Zygocystis cometa* Stein, are a result of the study of the Monocystid parasites occurring in the seminal vesicles of the earthworms found in the vicinity of St. Paul, Minnesota. *Monocystis agilis* Hesse, a parasite in the seminal vesicles of the earthworm *Lumbricus terrestris* L., is used as an example of the order Gregarinida in the laboratory work in parasitology at the University of Minnesota. An effort has been made for several years to find a local supply of *L. terrestris* that contained this parasite which might be used for laboratory purposes, but satisfactory material was never found and it has always been necessary to procure earthworms from other sources. At the suggestion of Dr. William A. Riley the writer undertook an examination of the earthworms found in this vicinity with the idea of determining whether or not *M. agilis* was to be found here, and if so, whether there was any periodicity in its presence in the earthworms. This work has extended over two years.

Three species of earthworms were found to be common here. The most abundant species is *Helodrilus caliginosus* var. *trapezoides* (Dugès). It is found in gardens, pastures, woods and compost heaps. The second most abundant species is *Lumbricus terrestris* L., and is found principally in and near compost heaps. The third species, *Helodrilus foetidus* (Savigny), has only been found in compost heaps and then not commonly. I am indebted to Dr. Frank Smith for the specific determination of the earthworms.

In the course of the two years, many specimens of *L. terrestris* have been collected and examined in the winter, spring, summer and fall months but not a single specimen of *M. agilis* has been found in any of them. It seems certain from the examinations that have been made that *L. terrestris* is free from infection of the parasite *M. agilis* in this region. Only a very few specimens of *H. foetidus* have been examined but no gregarines have been found in the seminal vesicles of any of them. On the other hand an examination of specimens of *H. caliginosus* var. *trapezoides* disclosed the fact that practically all mature individuals were very heavily infected with *Z. cometa*.

Individuals of the genus Zygocystis live in association during that part of the trophozoite stage in which they are free in the seminal

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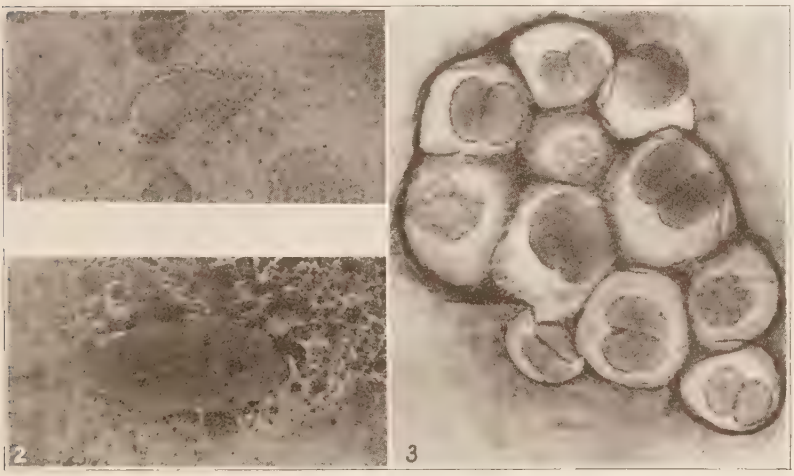
vesicles. In the case of *Z. cometa* the association usually consists of two individuals joined at their anterior ends, but occasionally three individuals may be joined in this way, and solitary individuals are sometimes found. The ectoplasm is thick and deeply furrowed; the ridges between the furrows continue beyond the posterior end of the body forming a tuft of long cilia, which is characteristic of the species (Fig. 2). The earliest known stage of *Z. cometa* in the seminal vesicles of the earthworm is that of the young trophozoite within the blastophore (the central part of the sperm morula consisting of residual protoplasm and surrounded by the spermatozoa). Here it grows and develops at the expense of the sperm morula (Fig. 1). The latter is finally destroyed by the trophozoite which then becomes free in the material of the seminal vesicles; two of the free trophozoites then become attached at their anterior ends and undergo considerable development in size.

Pfeiffer (1891) has suggested the possibility of more than one sporozoite of *Z. cometa* entering a single blastophore and developing there in association. In the intrablastophore stages that the writer has observed only solitary individuals have been seen. Solitary individuals of the parasite may occasionally be found free in the seminal vesicles, indicating that these have not yet formed an association with a second individual, rather than that an association has previously existed and later been broken. The writer is therefore convinced that normally two trophozoites of *Z. cometa* do not form an association until after they have become free in the seminal vesicles.

When the two attached trophozoites become mature and are ready for reproduction they secrete a common membrane around themselves. They are then known as gametocytes and still remain an association of two individuals (Fig. 3). Nuclear division takes place in each of the gametocytes until a very large number of small nuclei are formed, when, in turn, the cytoplasm is divided into a number of small masses, each of which surround one of the nuclei. These tiny nuclei with their surrounding cytoplasm are the gametes. The wall of each of the gametocytes is then dissolved and the gametes conjugate in pairs. The conjugation of the gametes forms a sporoblast. This becomes oval in shape, secretes a tough wall about itself and within it nuclear division again takes place to form eight sickle-shaped sporozoites. How these sporocysts containing the sporozoites are transferred from one earthworm to another causing a new infection is as yet unknown. It has been demonstrated by Hesse (1909) and others that the digestive juices of the earthworm dissolve the wall of the sporocyst and set the sporozoites free in the intestinal tract; but how the sporocysts reach the alimentary canal, and how the freed sporozoites reach the seminal vesicles is not definitely known.

Ruschhaupt (1885) and Pfeiffer (1891) have noticed a correlation between the development of the sexual glands of *L. terrestris* and the developmental stages of Monocystis. They have interpreted this as being a correlation between the seasons of the year and the developmental stages of the parasite. It has been pointed out by Hesse (1909), however, that the development of Monocystis is dependent on temperature and the degree of development of the host rather than on the seasons. In environments such as compost heaps temperature as a factor in development is almost independent of the seasons. The degree of development of the host is, in part, dependent on temperature and in compost heaps where temperature and other environmental conditions are favorable for



EXPLANATION OF FIGURES

Zygocystis cometa Stein in the seminal vesicles of the earthworm *Helodrilus caliginosus* var. *trapezoides* (Dugès)

Fig. 1.—Trophozoite within the blastophore of sperm morula. × 360.

Fig. 2.—Trophozoite free in the seminal vesicles. × 100.

Fig. 3.—Gametocytes in seminal vesicles. × 100.

the growth of earthworms during the entire year, earthworms are always present that are mature enough for the development of all stages of Monocystis.

In the investigation of *Z. cometa* specimens of *H. caliginosus* var. *trapezoides* have been dissected during all months of the year. The earthworms were collected for the most part in a compost heap. In two or three cases during the summer months the worms were collected from a low meadow. Regardless of the time of year, or the source of the specimens, the earthworms which possessed a well developed clitellum were always infected with *Z. cometa*. Usually trophozoites, gametocytes

and sporocysts could be found in a single specimen, and often in addition to these the trophozoite within the blastophore could be observed. This is so universally true that it is possible in this vicinity, at least, to collect this species of earthworm at any time of the year, even in the dead of winter, and find those with a well developed clitellum always infected with practically all stages of the parasite. Many earthworms were dissected which did not possess a well developed clitellum and in these the trophozoites, gametocytes and sporocysts of *Z. cometa* were very seldom found. The writer is convinced from these observations that in the case of this parasite there is no correlation whatever between the developmental stages and the seasons. It seems very evident that there is a correlation between the developmental stages of *Z. cometa* and the age, or the degree of development of the sexual organs, of its host *H. caliginosus* var. *trapezoides*. The degree of development of the sexual organs of the latter is dependent in part on the temperature of its environment.

As stated above three species of earthworms were found in the compost heap where the material for this study was obtained. On account of the exceeding abundance of *Z. cometa* in *H. caliginosus* var. *trapezoides* it is very likely that if it were not a specific parasite it would be found in the other species of earthworms from the same environment. A number of specimens of the other species were dissected and examined for the presence of this parasite in the seminal vesicles but none was ever found either in *L. terrestris* or in *H. foetidus*. During the examination of these two latter species no other gregarine was ever found in the seminal vesicles. Both of these species of earthworms seem to be free from Monocystis infection in this particular region. No other gregarine other than *Z. cometa* was found in the seminal vesicles of *H. caliginosus* var. *trapezoides*.

Hesse (1909) has shown that the sporozoites of Monocystis are freed from the sporocysts by the action of the intestinal juices on the wall of the sporocyst. He was unable, however, to follow the course of the sporozoites from the time they became free in the alimentary canal until they appeared in the seminal vesicles. It appears reasonable that if a sufficient number of sporozoites could be liberated in the digestive tract of an earthworm, and if the specimen could be fixed and sectioned shortly afterward, that some of them might be located and the course of their path to the seminal vesicles thereby discovered. With this in mind an agar preparation was prepared as follows: 0.75 gram of agar was dissolved in 50 cc. of water. To this was added 50 cc. of the filtrate of very rich soil mixed with water. The seminal vesicles of 100 earthworms heavily infected with the sporocysts of *Z. cometa* were added to this mixture. An examination of this medium showed it to

be very heavily impregnated with the sporocysts of the parasite. A number of earthworms which had been starved for forty-eight hours were placed in this medium and they lived for seven or eight days. At regular intervals they were dissected and the digestive tract examined for sporocysts and sporozoites of *Z. cometa* but without success. The earthworms moved freely in the agar medium but apparently did not ingest any of the material. Only two sporocysts were found during these examinations, one in the digestive tract of each of two earthworms. This does not seem to be a satisfactory method of infecting earthworms with this parasite.

SUMMARY

The seminal vesicles of specimens of *Lumbricus terrestris* L. and *Helodrilus foetidus* (Savigny) collected in the vicinity of St. Paul are not parasitized by *Monocystis agilis* Hesse, or by any other species of Monocystis. Specimens of *Helodrilus caliginosus* var. *trapezoides* (Dugès) collected in this region are very heavily infected with *Zygocystis cometa* Stein, but not with any other species of Monocystis.

There is no correlation between the seasons and the developmental stages of *Zygocystis cometa.* There is a correlation between the degree of development of the host, *Helodrilus caliginosus* var *trapezoides,* and the development of the parasite, *Zygocystis cometa.*

Multiple infection of the blastophores by the sporozoites of *Zygocystis cometa* does not normally occur. Agar impregnated with the sporocysts of *Zygocystis cometa* did not prove successful in infecting earthworms with this parasite.

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OBSERVATIONS ON *RHABDITIS HOMINIS* KOBAYASHI IN THE UNITED STATES*

J. H. Sandground

Rhabditis hominis was first recorded by Kobayashi (1920) from the stools of a number of school children in certain prefectures in Japan. The nematode was also found by Takiki (cited by Kobayashi, 1920) in another part of Japan in the stools of a small percentage of children who were examined for intestinal parasites. No further records of this worm either from Japan or any other country have to my knowledge appeared since, although in his original publication on *Rhabditis hominis* Kobayashi stated that its parasitological importance was to be a subject for future study.

In July and August, 1923, a number of specimens of human feces tentatively diagnosed as containing the free-living phases of *Strongyloides stercoralis Bavay,* were forwarded to our laboratory from Virginia and were brought to my attention for confirmation of the diagnosis and for use as study material. Another series of specimens, also stated to contain the larvae and free-living sexual individuals of *Strongyloides stercoralis* was received some time later from Georgia for the same purpose. In all cases the specimens were received in small tin or glass containers and their appearance presented no signs of any gross contamination such as with soil. All the specimens were at least forty-eight hours old when examined and smear preparations showed the presence in large numbers of a rhabditis-like nematode in stages of growth ranging from small larvae to sexually mature adults. It is easy to understand how these worms could be confused with the structurally similar rhabditiform generation of Strongyloides by one not specifically acquainted with the detailed morphology of the free-living generation of this genus. In addition to minor differences in the appearance of the worms that impressed me, the absence of filariform larvae from fecal specimens of this age suggested an error in the original diagnosis. More careful examination of the sexual adults permitted the identification of

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The author desires to acknowledge his indebtedness to Dr. N. A. Cobb, Burea of Plant Industries, Washington, D. C., for examining the material and for giving so freely of his valuable counsel; and to Dr. G. Steiner also of the Bureau of Plant Industry, for drawing our attention to the literature on the carriage of species of Rhabditis by flies. My hearty thanks are also due to Dr. W. W. Cort, Associate Professor of Helminthology in the Medical Zoology Department, for his kindly interest and suggestive criticism in the presentation of this work.

the organism as *Rhabditis hominis*. The worms coincided in all major criteria, such as the dimensions of the various organs and their relative lengths, and the structure of the bursa copulatrix, with the description of *R. hominis* Kobayashi (1920). However the lack of correspondence between description and figures of certain structures, such as the shape of the buccal cavity and the spicules and the non-representation of the gubernaculum in the male (Kobayashi 1920, Figs. 1 and 5) makes it advisable to present new and slightly more detailed drawings which I think will allow for the easier recognition of *R. hominis* and its differentiation from the hordes of species in this genus. (See Figs. 1-5.)

In addition to the points mentioned above, Dr. N. A. Cobb drew my attention to the presence in the male of an additional papilla which is not mentioned in Kobayashi's description and is absent from his figures. This papilla, uniformly present but inconspicuous especially when the worm is viewed in certain positions, is situated in the median ventral line 40 to 45μ anteriad to the proximal end of the spicules. Further study also revealed a second additional spicule located approximately 95μ anteriad to the end of the spicule (Fig. 1, *ap, pp*). It seems very possible that these papillae may have escaped the notice of Kobayashi in his material.

The fact that nine specimens of human feces containing *Rhabditis hominis* have within the past year been received in our laboratory after being diagnosed as *Strongyloides stercoralis* by experienced laboratory diagnosticians indicates the confusion that this organism may cause. In this connection the presumption by Kobayashi of the confusion between these two organisms that has occurred in certain Japanese records, seems entirely warranted (for cited references see Kobayashi, 1920). The idea prevails among many diagnosticians that the finding of sexually mature Rhabditis-like nematodes in human stools is per se a specific indication of an infection with *Strongyloides stercoralis* of Leichtenstern's tropical variety. It is now evident that such a generalization is not valid.

Rhabditis hominis in the light of the information that is to follow seems to enjoy a considerable range of distribution and because of the frequency of its occurrence in feces, it becomes necessary to distinguish between it and Strongyloides. For this purpose a differential comparison between the salient features of the morphology of the two forms is presented in tabular form below (Table 1). In fresh feces, the finding of rhabditiform larvae necessitates for a specific identification the differentiation between Strongyloides and *R. hominis*. But if the material is more than a day old, conditions are frequently such that any eggs of hookworms that may have been present would have developed and hatched, and it then might be necessary to distinguish between three

forms of rhabditiform larvae. As is well known, the shape and size of such larvae is so similar that for their differentiation recourse to the relative size of the buccal cavity and the genital primordium is required. The buccal cavity of the rhabditiform larva of the hookworms *Necator americanus* and *Ancylostoma duodenale* is 10 to 12μ long or roughly, as long as the body is wide at the level of the base of the buccal cavity. The buccal cavity of the corresponding larva of *Strongyloides stercoralis* is 8 to 10μ long and is shorter than the body is wide at the level of the base of the buccal cavity. The buccal cavity of the rhabditiform larvae of *Rhabditis hominis* is 15 to 19μ long or longer than the body is wide

TABLE 1.—*A Differential Comparison of the Morphology of Rhabditis hominis and the Free-Living Phases of Strongyloides stercoralis*

Rhabditis hominis	*Strongyloides stercoralis*
MALE	MALE
Dimensions: 0.9 — 1.3 mm. long 0.03 — 0.05 mm. broad	Dimensions: 0.7 — 0.9 mm. long 0.035 — 0.04 mm. broad
Buccal Cavity: 20μ long	Buccal Cavity: 13μ long
Bursa Copulatrix: present although often inconspicuous	Bursa Copulatrix: absent
FEMALE	FEMALE
Dimensions: 1.4 — 2 mm. long 0.12 mm. broad	Dimensions: 1 — 1.2 mm. long 0.05 mm. broad
Buccal Cavity: same as in male	Buccal Cavity: same as in male
Reproduction: ovo-viviparous	Reproduction: usually oviparous
Eggs: 24 — 44μ by 32 — 28μ; often arranged in a double row in each uterus; 20 — 50 in number	Eggs: 42 — 46μ by 36 — 33μ; usually arranged in a single row in each uterus, 16 — 18 in number
LARVA (Young rhabditiform)	LARVA (Young rhabditiform)
Dimensions: 0.24 to 0.3 mm. long 0.12 to 0.03 mm. broad	Dimensions: 0.2 — 0.25 mm. long 0.016 mm. broad
Buccal Cavity: 15 — 19μ long	Bucal Cavity: 8 — 10μ long
Genital Primordium: 22 to 24μ long	Genital Primordium: 34 — 36μ long
This larva always develops into a rhabditiform sexual adult	This larva develops either into the sexual intermediate rhabditiform generation or metamorphoses directly into the filariform larva

at the level of the base of the buccal cavity. Under suitable conditions the rhabditiform larvae of the hookworm metamorphose into the characteristic sheathed filariform larvae in about ninety-six hours, while in less than forty-eight hours the rhabditiform larvae of Strongyloides either develop into the sexual intermediate generation or metamorphose directly into the characteristic slender filariform larvae. In the case of *Rhabditis hominis* the rhabditiform larvae always develop to sexual maturity and no filariform larval stage is produced. This then presents a simple and expedient method of differentiating between *R. hominis,* Strongyloides and the hookworms.

The foregoing consideration will serve to show that the differentiation of the two forms is not an easy matter unless the organism be critically examined in one or several stages of its development. It also raises the question of the reliability of the information that is available on the incidence of Strongyloides infections in man in this country. There can be little doubt that the figure supplied by Brumpt (1922:697) on the incidence of *S. stercoralis* in the State of Georgia, namely, 20.5 per cent. is much too high. A hookworm and general helminth survey recently carried out by the writer in a rural community in southern Georgia revealed only a single case of Strongyloides infection in an examination by smear and culture methods of over 500 children. This low index of infection with *S. stercoralis* appears to be general throughout the state. Official data amply support the contention that the index of infection with this parasite is low, probably under 1 per cent., even in those localities where the hookworm index is relatively high. Thus the annual report of the Georgia State Board of Health for 1923 shows five cases of Strongyloides in a total of 6,261 stool examinations, an incidence of 0.08 per cent. However, Moss (1922) in a survey of 597 cases of the late war veterans from Southern States found a higher index for Strongyloides, namely, a fraction over 4 per cent.

The possibility of spurious diagnoses may also perhaps throw some light on the numerous accounts to be found in the literature on the successful treatment of Strongyloides infections with such drugs as male fern, chenopodium, carbon tetrachloride, etc., in as much as in authentic cases of Strongyloides infections these drugs are not at all efficacious.

A number of considerations have forced the conclusion that the finding of *Rhabditis hominis* is a consequence of contamination and that the organism is in no sense a parasite of man. In the first place it became very evident early in my experience with the worm that it could easily be cultured in a variety of media, a phenomenon unparalleled in the entire range of nematode parasites of the higher animals. The worms thrive and multiply prolifically in a mixture of charcoal and feces, on plain and nutrient agar plates, and in fact on most media in which moderate putrefaction is occurring. A gravid female placed in a petri dish culture with just sufficient water to prevent desiccation and kept at room temperature (20° to 25° C.) will produce a new generation in as short a time as forty-eight hours. The worms have been maintained in culture in our laboratory for over five months. It may be of interest to note that the larva constitutes the most resistant stage in the life cycle. In one instance, a culture inadvertantly dried leaving a large number of coiled quiescent larvae, which on transplantation to a new culture medium some two months later immediately revived and developed to maturity.

In addition to finding *Rhabditis hominis* in the feces of man, the feces of a number of animals, namely: dogs, rabbits and rats which were periodically cultured in connection with our Strongyloides studies have at various times yielded the same organism. It was significant, however, that the worms were not regularly present in fecal cultures that were made from these animals. This could only be interpreted in one of two ways; the organisms were either incidental and temporary parasites in the alimentary tracts of these various animals or otherwise their presence in feces was to be explained as due to the contamination of the material by the ubiquitous free-living nematodes after its deposition. From the title of Kobayashi's paper it could be inferred that the worms actually established themselves, even though perhaps temporarily, in the human alimentary canal and were actually found in that situs but no evidence in support of this inference is to be found in the text of his paper. Kobayashi stated that he found the worms in large numbers in freshly passed stools after having taken strict precautions to preclude the entry of free-living nematodes. In his cases, however, the organisms disappeared spontaneously from infected individuals (that is, without the administration of vermifugal treatment) since they could no longer be demonstrated when the feces of these individuals were re-examined some two or three months later. Kobayashi concluded from these observations that *Rhabditis hominis,* although it may not be a true and permanent parasite, once having obtained lodgement in the alimentary canal can nevertheless maintain itself and thrive in that habitat.

In our own experience re-examination of the feces of those animals from which I had previously isolated *Rhabditis hominis* have uniformly failed to yield the worms when rigidly strict measures to prevent contamination were employed in the collection of the feces. This is compatible only with the view that the organisms were found solely as a result of the contamination of the excrement from the floors of the animal cages or from some other extraneous source.

Further evidence supporting the contention that *R. hominis* cannot establish itself as an inhabitant of the intestine was forthcoming from my experiences in Georgia during the summer of 1924. Of the many hundreds of fecal specimens examined, three were found to contain rhabditiform larvae which proved after culturing to be *R. hominis.* In one of these three specimens contamination with soil was evident and it was obvious that this contamination had occurred at the time of filling the specimen container after passage. In only one of these three cases was it practicable to visit the donor of the material and to obtain a second specimen. The subject in this instance was a fourteen year old negro boy. The second specimen was obtained only two days after the first but was collected under personal instructions to avoid any

contamination. The specimen when examined by smear and culture methods, using the very efficient Baermann technique of isolation (for a description of which see Cort et al., 1922), was free from all nematodes. A third specimen from this same individual obtained ten days later under precautionary conditions was again negative. It may be mentioned here that in two further specimens that I was able to obtain from our original nine human cases the organisms also could not be found.

In order to establish further my contention that *R. hominis* cannot adapt itself to the abnormal environment presented by the alimentary canal of man, a series of experiments were instituted to procure if possible human infections by the direct ingestion of the worms. The material used for these experiments was derived from cultures of *R. hominis* obtained from human cases. Adult worms from fecal cultures were isolated and grown in an agar medium. Since *Rhabditis hominis* is viviparous, it is only possible to use larvae of various ages and mature worms as material for infection. Intestinal parasitic nematodes obtain entrance to their host in one of two ways: they are either introduced into the mouth as eggs or larvae or they are able in a special infective larval stage to penetrate the skin or mucous surface and after a migration through the system finally become established in the alimentary canal. In my experiments both methods were employed and having found justification in the total absence of clinical symptoms in Kobayashi's as well as my own cases, relatively large numbers of the organisms were used.

ATTEMPTS TO PROCURE INFECTION BY MOUTH

Only July 21, 1924, about 200 active larvae in the second stage of development (i. e., after the first ecdysis) were collected from the lid of a petri dish onto which they had migrated from a culture and were swallowed with a little water. Stools were collected daily under conditions to prevent contamination and were examined both by smear and culture methods for a period of fourteen days after the ingestion of the larvae. No evidence of infection was obtained during this period. On August 5, a second attempt to procure infection by mouth was made. On this occasion more than 1,000 worms in stages of development ranging from young larvae to sexually mature adults were isolated from a culture and swallowed. Stools were rigorously examined and cultured for nine days subsequently but again with no evidence of infection forthcoming.

ATTEMPTS TO PRODUCE INFECTION BY SKIN PENETRATION

On August 17, 18, 19, respectively, about 400, 300 and 600 actively motile larvae of varying stages of development were collected in a

shallow watch glass and placed on the forearm until all moisture had disappeared. There seemed to be some sensations of irritation or itching in the area where the larvae were applied but these were probably subjective. There was no evidence of an erythema. On moistening and scraping the skin a few minutes later a number of larvae could be found but these were already dead. The stools watched carefully for ten days were negative. Before I finally ruled out the possibility of inducing an infection by ingesting the worms, one more attempt was made. On October 10, between 400 and 500 of the quiescent resistant larvae that had remained in a desiccated culture were revived with water and swallowed. The stools were examined daily again for eighteen days and failed to yield any evidence even of the survival of the ingested larvae. An attempt to infect a negro volunteer, 14 years of age by placing 500 and the next day roughly 1,000 larvae on the skin of the forearm and examining the stools by culturing for eight days was also negative. In summing up the results of these attempts I may say that after numerous attempts to induce infection, both by ingesting the larvae and giving them an opportunity to penetrate the skin, the stools were examined at frequent intervals for a total period of nearly four months without any evidence of the possibility of procuring infection by these methods being obtained.

This lack of success makes it appear justifiable to assume that the worms are introduced into the feces in the period that intervenes between the passage of the stool and its examination. If this period be a protracted one, the worms will have an opportunity to multiply and consequently will be found in large numbers. The stools when submitted for examination by an ignorant rural population is often deposited on the ground and is transfered to the container by the use of a twig or any other convenient implement. At this point contamination is easily possible.

The probability of *R. hominis* being normally an inhabitant of the soil is increased by illuminating evidence submitted by Dr. N. A. Cobb in a letter acknowledging receipt of some of our material for his examination and opinion. It appears that Cobb's attention had already been drawn to this organism which pending the presentation of an opportunity of examining and comparing some of the type material he tentatively preferred to regard as a new species. The following extracts of his letter are published with Dr. Cobb's kind permission: "The only previous record of this species dates back about five years and the specimens were found in sand carrying fly puparia from the United States Marine Hospital experiments at Wilmington, North Carolina. . . . I made many experiments in an effort to infect fly larvae and pupae without success. When, however, the larvae or pupae

were artificially injured slightly, it was easier to infect them." Cobb in conversation with the writer further stated that the species might also be obtained in abundance by burying meat or other suitable bait in the soil.

In addition to the possibility of the worms entering the feces from the soil there also remains a possibility of their being introduced by various filth flies. That contamination of this order occurs was evidenced by the frequency with which fly larvae were found in a number of specimens submitted for examination in the field this summer. A number of papers incriminating flies in the carriage and transference of free-living nematodes have appeared in the literature. Thus Menzel (1923) in a recent paper demonstrated that an unidentified species of Rhabditis which he found in feces is able to adhere in both the larval and adult stages to the bodies of house flies, and in this way is transported to other situations where the fly may alight. Aubertot (1923) also showed that *Rhabditis pellio* (Schneider) is able to pass uninjured through the alimentary tract of the fly Drosophila.

As yet I have not been able to test experimentally this very feasable mode of transportation for *Rhabditis hominis,* but it might be well to take into consideration the possibility of flies being responsible for the introduction of free-living nematodes into feces and, as a precaution, measures should be taken to insure against such contamination.

SUMMARY

1. A species, probably the same as *Rhabditis hominis* as described by Kobayashi from the stools of children in Japan, is recorded for the first time from America.

2. The nematodes were found in nine specimens of human feces from Virginia and Georgia and were originally diagnosed as *Strongyloides stercoralis* by experienced diagnosticians. Because of the confusion of these two organisms, it seems probable that the incidence of Strongyloides is not so high as has hitherto been considered.

3. The same organism has also been found in the feces of dogs, rabbits and rats but, as is also the case with man, it is not found when precautionary measures to prevent the entry of free-living are observed in the collection of the feces. The worms are easily cultured in a mixture of feces and charcoal and in other putrifying media and multiply prolifically for an apparently indefinite time.

4. Repeated attempts to infect the human subject with larvae have failed. This and other evidence furnished indicates that *Rhabditis hominis* cannot even temporarily establish itself as a parasite in the alimentary canal. The worm is therefore to be regarded as a free-living coprophagous species which may appear in the feces as a result

of contamination with soil or is possibly introduced into the feces by the visits of filth flies.

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Abbreviations

t Testis
ap Anterior medio-ventral papilla
pp Posterior medio-ventral papilla
g Gubernaculum
bp Bursal papillae
s Spicules
bc Buccal cavity
ge Genital primordium
v Vulva

Explanation of Plate XXII

All figures are camera lucida drawings of living material. The scale at the side of Figures 1 to 4 equals 20μ, that in Figure 5 equals 100μ.

Fig. 1.—Adult male.

Fig. 2.—Anterior portion of an adult worm much enlarged.

Fig. 3.—Bursa copulatrix of male showing arrangement of the supporting papillae.

Fig. 4.—Young rhabditiform larva.

Fig. 5.—Young female.

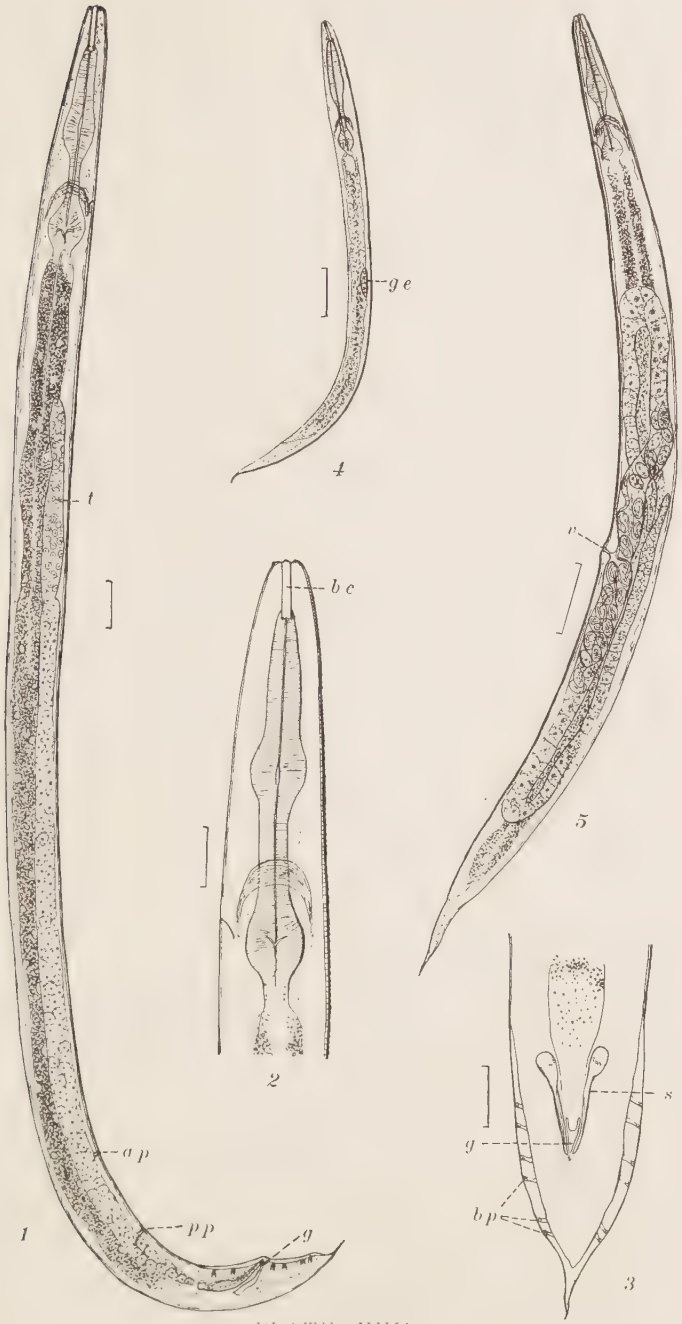

PLATE XXII

A CASE OF HUMAN MYIASIS CAUSED BY THE OX-WARBLE, *HYPODERMA BOVIS* DE G.

W. B. Herms
University of California

The wandering habit of certain species of Oestrid fly larvae in the body of the host is well illustrated in the case of a young man who reported (Feb. 4, 1925) to the writer the day following the emergence of a larva. The history of the case is essentially as follows:

Mr. C. is a ranch superintendent spending much of his time on horseback. On a Sunday toward the end of July, 1924, he had ridden to a point known as Mission Ridge near San José, when feeling tired, he dismounted and lay down on the ground in the open and slept. He distinctly remembers that his shirt had rolled up above his belt exposing his skin but felt no irritation at the time. Whether this exposure was taken advantage of by the fly can only be a matter of conjecture. Several days later, exact time not remembered, soreness was experienced and a slight swelling in the region of the right groin appeared. In about a week the swelling had increased to the width of a hand with no discoloration. The swelling then crept downward toward the left side affecting the scrotum, thence downward along the left leg to the knee and calf, thence back up the left leg following about the same course to the left groin, thence across to the right groin and back again to the left and upward along the left side of the body, slightly anterior to the shoulder, thence downward to the upper right arm to near the elbow, when the arm could not be raised without great pain, thence the swelling travelled upward again to the neighborhood of the shoulder blade where a "hive-like" local swelling was formed without any itching sensation. Mr. C. stated that at this point he was "bothered" all night and while rubbing his arm and manipulating his shoulder muscles a larva of some insect "popped" out. This emergence took place about the end of October (1924). The larva was placed in a vial for shipment but was lost in transit. Mr. C. is a graduate of Stanford University with good entomological training and his statements can be relied on.

Relative to the second larva which was delivered to the writer in person on the day following its emergence, Mr. C. states that since October when the first larva emerged, no further swellings were observed, but soreness in the region of the thigh and lower abdomen, similar to severe strain, persisted. However, on January 28 (1925) he experienced a severe "soreness" in the region of his right thigh which gave much distress, particularly when walking. By that night a swelling

had developed and the following day the muscular soreness continued to spread; by January 31 a hernia-like swelling had developed which enlarged upward and outward to the region of the belt-line, the lower hernia-like swelling gradually disappearing. Sunday night, February 1, a hive-like swelling as observed in the case of the first larva began to form, enlarging to an area of about 4 by 8 inches. Tuesday evening, February 3, lymph exuded from a small opening near the middle of the swollen area. About a tablespoonful of lymph stained with blood was pressed out and in the process of manipulation a larva similar to the first "popped" out. This specimen was delivered to the writer February 4 in good condition and identified by means of characters given by Laake (1921) and Warburton (1922) as a third-stage larva of *Hypoderma bovis* De G. The larva was milky white in color, about 12 mm. in length by 2 mm. in width at the middle, tapering bluntly at both ends. Very little swelling and practically no discoloration were visible on examination, although the point of emergence was clearly seen.

Mr. C. states that he was greatly handicapped during the entire time that the first larva was migrating, feeling very logy and sore, slowing him up considerably during the important season of fruit picking. He was greatly relieved when the larva was finally eliminated and did not suspect the presence of the second one until the time already stated.

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THE INGESTION OF ERYTHROCYTES BY *TRICHOMONAS HOMINIS* AND ITS OCCURRENCE IN THE PUS OF AN AMOEBIC LIVER ABSCESS*

JOHN F. KESSEL

The question of the pathogenicity of Trichomonas in man has in the past been open to discussion and the present tendency (Hegner and Taliaferro, 1924) resulting from the observations by a number of workers on the ingestion of erythrocytes by Pentatrichomonas and its presence in cases of long standing diarrhoea (Chatterjee 1917, Haughwout and de Leon 1919, and Kofoid and Swezy 1923) is to differentiate between *Trichomonas hominis* which is usually described as possessing four anterior flagella and *Pentatrichomonas ardin-delteli* which has five anterior flagella, and to assign the rôle of pathogenicity to Pentatrichomonas.

While the observations here recorded do not disprove this hypothesis they are of interest in suggesting the possibility that *T. hominis* may adapt itself to a pathogenic environment.

1. Observations made in the Parasitology Laboratory, Peking Union Medical College: (a) *Trichomonas hominis* found in the stool of a patient suffering from bacillary dysentery and (b) *Trichomonas hominis* (*vaginalis?*) from the urine of another patient passed at the time of menstruation were found to contain ingested red blood corpuscles. The flagellar count was made by the aid of an oil immersion objective and showed four anterior flagella with the characteristic arrangement usually described for *Trichomonas hominis*.

2. While the writer was carrying on a survey of the human intestinal protozoa in Seoul, Korea, in August, 1924, Dr. A. I. Ludlow of the Severance Union Medical College, operated on a case of amoebic liver abscess in a Korean and some of the pus obtained from this case was examined for amoeba.

A brief history of the case is as follows: Native male Korean; age, 30; occupation, farmer. Important diseases of childhood, measles and malaria. No history of dysentery. Present trouble began nine months ago with pain in the right hypochondrium. A Korean physician operated and between that and the later operation the patient discharged pus through a small artificial fistula. Dr. Ludlow operated August 22, 1924, making an incision over site of the old operation. Large cavity found in pleura which led to liver: 500 c.c. of red pus and sloughing tissue were removed at operation.

* From the Parasitological Laboratory, Department of Pathology, Union Medical College, Peking, China.

Microscopic examination of the pus showed numerous, actively motile forms of *Endamoeba dysenteriae* containing ingested red blood corpuscles. In addition to this protozoön, numerous actively motile *Trichomonas hominis* were present in the pus. A careful flagellar count revealed three anterior flagella in one clump and a fourth, slightly separated from these three. The constant presence of four anterior flagella thus serves to separate this organism from Pentatrichomonas. The undulating membrane was characteristic of *Trichomonas hominis.* None of the flagellates were observed to contain ingested red blood corpuscles. Fecal examination of the patient revealed cysts of *Endamoeba dysenteriae,* of *Giardia intestinalis,* and motile forms of *Trichomonas hominis.*

Haughwout and de Leon (1919) report a trichomonad in exudate removed by aspiration from the pleural cavity of man in the Philippines but offer no explanation as to how the organism gained entrance to the pleural cavity. The writer is not aware of a previous report of *Trichomonas hominis* from the pus of an amoebic liver abscess and considers such a finding worthy of note in that it indicates the possibility that *Trichomonas hominis* passed unharmed through the blood from the capillaries of the intestine to the liver and that it demonstrates the ability of this organism to live successfully within the mammalian host under pathological conditions of an amoebic liver abscess.

These observations do not indicate that *Trichomonas hominis* is (*per se*) able to produce pathological conditions in man, for in the cases in which red blood corpuscles were observed to be ingested by the flagellate, there were other causes for the presence of the free corpuscles and Trichomonas merely demonstrated its ability to ingest this particular type of food which was at hand. Further, in the case of the amoebic liver abscess Trichomonas was found in association with *E. dysenteriae* which was probably responsible for making the primary invasion into the capillaries of the intestine and thus preparing an entrance through which the Trichomonas later passed into the blood stream.

However, the ingestion of red blood corpuscles by *T. hominis* and its association with the amoebic abscess do indicate a possibility that under suitable conditions, this flagellate may become pathogenic.

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THE EOSIN-CRITERION OF THE VIABILITY OF PROTOZOAN CYSTS AS APPLIED TO CYSTS OF *HARTMANNELLA HYALINA* TREATED WITH CHLORIN-WATER *

JOHN F. KESSEL

Owing to the fact that no satisfactory method has been developed for the excystment and culture of the amoebae parasitic in the human intestinal tract no absolute criterion has been established to distinguish the viable from the nonviable cysts.

Kuenen and Swellengrebel (1913), in working with cysts of *Endamoeba dysenteriae*, noted that eosin penetrated some of the cysts and stained them red while other cysts were resistant to the eosin. They concluded that those cysts which colored red were dead cysts and those which did not take the stain were viable.

Later workers have used this method as a criterion of life and death, though Wenyon and O'Connor (1917) suggested that not all unstained cysts are viable. Yoshida (1920) and Boeck (1921) described a plasmolysis in cysts of the intestinal Protozoa and noted that though the structure of the cysts was degenerate and in some cases had completely disappeared, the anilin dye did not penetrate. The conclusion was that all cysts which take the eosin stain are dead but that all dead cysts do not absorb this stain. Cutler (1920) in working with the cysts of soil Protozoa was able to induce unstained cysts to excyst but was unable to produce excystation with cysts which had stained. He does not prove, however, that all green cysts are viable.

The present problem was undertaken to determine the percentage of free chlorin in water necessary to retard development of cysts of *Hartmannella hyalina* and data were kept at the same time which are of interest with reference to the eosin-criterion of viability of Protozoan cysts.

MATERIALS AND METHODS

Cysts of the amoeba in question were recovered from a sample of human feces which has been used as a control in experimenting on the resistance of cysts of the human intestinal Protozoa to various disinfectants. The motile forms and the cysts of this amoeba answer to the description and figures of *Hartmannella hyalina* given by Dobell and O'Connor (1921).

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A stock medium consisting of 10 per cent. horse serum in Locke's solution having a p_H of 7.4 diluted with 50 per cent. distilled water was used as a culture medium because maximum excystment and growth did not occur when cysts were transferred to tap water or to distilled water. There was no difficulty in procuring numerous cysts from old cultures from which the solution had for the most part been allowed to evaporate. Chlorin-water of a known concentration, prepared by placing large amounts of chlorinated lime in water, was diluted to different concentrations with distilled water and placed in small stender dishes.[1] Cysts were then placed in the diluted concentrations for a period of ten minutes at which time they were transferred to the culture medium, which was also in small stender dishes. Ten minutes was chosen as an arbitrary time for the cysts to remain in the chlorin-water because it is the average time foreigners in China allow fruit and vegetables to remain in chlorinated-lime-water for the purposes of disinfection, and it is hoped that a careful comparison of the resistance of cysts of the intestinal Protozoa with the cysts of the coprozoic form under consideration may give important information regarding the value of this and other methods employed in washing fruit and vegetables.

At the time the cysts were transferred from the chlorin-water to the culture medium, a smear was made in iodine-eosin solution and the staining reaction of the first hundred cysts encountered was noted. Cysts were counted at this time in the stender dishes, the *number of cysts at transfer* in the tables denoting the average number of cysts present in six or more fields of the microscope. At the end of twenty-four, forty-eight and seventy-two hours, respectively, the cultures were examined and similar counts made for the cysts and motile forms. Smears were made on each successive day and the staining reaction of the cysts that remained was noted. As the motile amoebae which had appeared in the most favorable cultures began to encyst on the fourth day, readings were not continued after the third day, except in some cases where no motile forms had appeared.

In all sixty-four tests were made in water which contained from 0.006 per cent. to 4 per cent. of free chlorin. Where series of tests overlapped or experiments were repeated, the averages of single concentrations are shown. It should be noted that there was some variation in the resistance of different lots of cysts, the maximum percentage of free chlorin in water at which growth was obtained being 1 per cent. in one

1. The writer is indebted to Mr. John Cameron, Pharmacist, Peking Union Medical College, for preparing the concentrated chlorin-water and for determining the percentage of free chlorin present each time a series of experiments was begun

TABLE 1.—*Results of Transferring Cysts of* Hartmannella Hyalina *to Culture Medium After Ten Minutes in Chlorin-Water*

Percent of Free Chlorin in Water	Color of Cysts at Transfer	Number of Cysts per Field at Transfer	Results of Daily Examinations: 24 Hours — Cysts per Field	24 Hours — Motile Forms per Field	24 Hours — Color of Cysts* % G	% P	% R	48 Hours — Cysts per Field	48 Hours — Motile Forms per Field	48 Hours — Color of Cysts % G	% P	% R	72 Hours — Cysts per Field	72 Hours — Motile Forms per Field	72 Hours — Color of Cysts % G	% P	% R	96 Hours
4.0	All cysts were green at time of transfer to culture medium	32	32	0	0	40	60	32	0	0	10	90	32	0	0	5	95	Motile forms began to re-encyst on fourth day so the experiments were concluded
3.0		37	37	0	5	25	70	37	0	0	5	95	37	0	0	5	95	
2.5		26	26	0	15	15	70	26	0	5	10	85	26	0	0	5	95	
2.2		29	29	0	20	20	60	29	0	5	10	85	29	0	0	10	90	
2.0		40	40	0	30	30	40	39	1	5	15	80	35	4	0	15	85	
1.8		35	35	0	50	20	30	35	0	40	33	27	29	6	0	25	75	
1.6		28	28	0	60	15	25	26	2	50	20	30	22	5	0	20	80	
1.4		20	20	0	55	20	25	17	3	40	30	30	15	8	0	30	70	
1.2		30	29	1	70	10	20	26	4	45	15	40	19	17	0	15	85	
1.0		30	28	2	79	0	21	27	3	41	0	59	21	7	0	0	100	
0.8		20	18	2	73	0	27	15	18	58	0	42	14	19	0	0	100	
0.6		25	21	4	75	0	25	15	25	64	0	36	14	40	0	0	100	
0.4		27	22	5	83	0	17	8	30	50	0	50	5	100	0	0	100	
0.2		36	8	28	100	0	0	5	200+	30	0	70	3	200	0	0	100	
0.1		30	2	26	100	0	0	0	250+	0	0	0	0	250+	0	0	0	
0.08		25	3	22	100	0	0	2	80	0	0	0	0	100+	0	0	0	
0.05		18	1	17	0	0	0	0	70	0	0	0	0	75	0	0	0	
0.03		23	2	21	0	0	0	0	65	0	0	0	0	80	0	0	0	
0.006		80	3	18	100	0	0	0	80	0	0	0	0	80	0	0	0	
Control		85	5	80	100	0	0	4	100	100	0	0	0	100+	0	0	0	

* G, green; P, plasmolized; R, red.

TABLE 2.—*Results of Transferring Cysts of* Hartmannella Hyalina *to Culture Medium After 24, 48 and 96 Hours in Chlorin-Water*

Percent. of Free Chlorin in Water	Condition of Cysts at Transfer* % G	% P	% R	Time in Chlorin Before Transfer,	Results After Transfer to Culture Medium: 24 Hours — Cysts % G	% P	% R	Motile	48 Hours — Cysts % G	% P	% R	Motile	72 Hours — Cysts % G	% P	% R	Motile
0.5	0	Comp. 100†	0	24 hours	0	Comp. 100	0	0	0	Comp. 100	0	0	0	Comp. 100	0	0
0.4	0	Part. 100†	0	24 hours	0	Part. 100	0	0	0	Part. 100	0	0	0	100	0	0
0.3	0	Part. 100	0	24 hours	0	Part. 100	0	0	0	Part. 100	0	0	0	100	0	0
0.2	5	Part. 95	0	24 hours	5	Part. 95	0	0	5	Part. 95	0	0	0	95	5	0
0.15	10	Part. 90	0	48 hours	5	Part. 90	5	0	3	Part. 90	7	0	0	90	10	0
0.1	20	Part. 80	0	48 hours	10	Part. 80	10	0	7	Part. 80	13	0	0	80	20	0
0.08	30	Part. 60	10	48 hours	15	Part. 60	25	0	10	60	30	0	0	60	40	0
0.06	40	Part. 40	20	48 hours	20	Part. 40	40	0	15	40	45	3	0	40	60	5
0.03	80	0	20	48 hours	45	Part. 10	45	5	30	10	60	12	0	10	90	30
0.009	90	0	10	48 hours	50	0	50	10	20	0	80	20	0	0	100	90
0.006	100	0	0	48 hours	55	0	45	50	30	0	70	50	0	0	100	100
0.15	0	Comp. 100	0	96 hours	0	Comp. 100	0	0	0	Comp. 100	0	0	0	Comp. 100	0	0
0.1	0	Part. 100	0	96 hours	0	Part. 100	0	0	0	Part. 100	0	0	0	Part. 100	0	0
0.06	10	60	30	96 hours	10	Part. 60	30	0	5	Part. 60	35	0	0	55	45	0
0.009	30	30	40	96 hours	20	Part. 30	50	0	15	Part. 20	65	3	0	Part. 20	80	3
0.006	50	15	35	96 hours	35	15	30	25	20	Part. 15	65	50	0	0	100	100

* G, green; P, plasmolized; R, red, † Comp., complete; Part., partial.

series, 1.5 per cent. in another and 2 per cent. at another time. Probably the age of the cysts and the moisture condition in the environment from which they were recovered are important factors in determining resistance.

The results in the accompanying tables are chosen as being representative of the series of experiments, and the following conclusions may be drawn:

DISCUSSION OF TABLES AND CONCLUSIONS

1. Two per cent. of free chlorin in water is the highest concentration at which excystment and development of motile amoebae was procured (Table I).

2. Maximum development was obtained from cysts that had been subjected to chlorin-water which ranged from 0.08 per cent. to 0.4 per cent (Table I). It is suggested that the rapid growth of bacteria in the medium to which cysts were transferred from concentrations below 0.1 per cent. of chlorin-water is responsible for the retarded development of the amoebae under these conditions. Nearly 100 per cent. excystment occurred but rapid multiplication of the amoebae did not follow. This condition held true in all controls.

3. Incomplete excystment and no excystment in transfers from the solution of higher concentrations is regarded as being the result of the action of the chlorin on the cysts (Tables I and II).

4. All cysts which were green and apparently normal at the time of transfer to the culture medium did not develop into amoebae, but following a certain interval, which varied from a few hours to seventy-two hours, those cysts which failed to develop either presented a typical condition of plasmolysis or else eventually stained red with the eosin (Table I, 1 per cent. and Table II, 0.03 per cent. for forty-eight hours).

5. This interval varies according to the concentration of the chlorin water used (Table I, 4 per cent. and Table II, 0.009 per cent.) and probably also varies according to the resistance of the cysts in question and according to the species. It should be considered in future experimental work on the resistance of Protozoan cysts where the eosin-criterion is used for determining their viability.

Boeck (1921) found that certain of the cysts which had undergone plasmolysis did not take the eosin stain though kept under observation for several months. In the present investigation observation on this point has not been continued for more than a month but a similar generalization may be drawn, i. e., only a small percentage of the cysts which have undergone plasmolysis will eventually be penetrated by the eosin. It should be noted, however, that the plasmolyzed cysts present a yellow appearance in the iodine-eosin rather than the characteristic

light green color of the viable cysts. It is further concluded that cysts, the cytoplasm of which undergoes a very pronounced shrinkage, the plasmolysis of which in this paper has been described as "complete," are less likely to take the eosin stain (Table II, 0.5 per cent.) than those which undergo a partial or gradual plasmolysis (Table II, 0.06 per cent. and 0.009 per cent., seventy-two hours). Complete plasmolysis has been noted to occur most commonly as the result of exposure to the higher concentrations of chlorin water (Table II, 0.5 per cent.) or to lower concentrations for extended periods of time (Table II, 1 per cent., ninety-six hours).

6. Amoebae failed to develop from cultures, the cysts of which stained red with the eosin or else presented a condition of plasmolysis (Table I, 2.5 per cent.). It is thus concluded that plasmolyzed cysts and cysts which stain red are incapable of development.

7. Providing free chlorin is not permitted to escape from the water, cysts subjected to chlorin-water for long periods of time show a greater mortality than those subjected for short periods of time (Table I, 0.05 per cent. and Table II, 0.06 per cent.).

SUMMARY

Conclusions in regard to applying iodine-eosin stain to Protozoan cysts:

1. Red cysts and plasmolyzed cysts are incapable of development.

2. All green cysts are not viable but a percentage of them may, after an interval, stain red or present a condition of plasmolysis.

3. This interval should be considered in future work where the eosin-criterion is used to determine the viability of cysts.

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TWO NEW CASES OF HUMAN CREEPING DISEASE (GNATHOSTOMIASIS) IN CHINA, WITH A NOTE ON THE INFECTION IN RESERVOIR HOSTS IN THE CHINA AREA

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From the medical and zoological points of view it is of especial interest that the nematodes of the genus Gnathostoma, which are naturally found in various animals, occur occasionally in man, causing disease. Five indisputable cases of Gnathostoma in man have hitherto been reported and all are from eastern Asia.

The first case was reported from Siam by Levinsen (1887) who studied a specimen collected by Dr. Deuntzer from the abscess of a native woman and described it as *Cheiracanthus siamensis* n. sp. This was an immature female of Gnathostoma. The second case was that of Dr. Kerr in Siam who removed a parasite from the cutaneous node of a native. The parasite was studied by Leiper (1909) who stated that it was an immature male, identical with that of Levinsen, and must be referred to the genus Gnathostoma with the specific name *G. siamense* (Levinsen). Later, Leiper (1911, 1913) discovered that *G. siamense* was synonymous with *G. spinigerum* Owen (1836), a view which is now generally accepted. The above-mentioned cases, therefore, are concerned with *G. spinigerum*. The parasite of the third case was obtained by Samy (1918) in the Malay States from a thenar abscess of a Chinese coolie and also referred to *G. spinigerum*. The fourth case was reported by Tamura (1919) in Japan. He treated a typical "creeping disease" of a Japanese woman just returning from a long sojourn in China, and removed a parasite which he referred to *G. siamense* (=*G. spinigerum*). The fifth case, was indigenous to Japan and has recently been reported by one of us (Morishita, 1923, 1924). The patient was a male Japanese, 43 years old, who had lived long in Tokyo. In 1923 he had "creeping disease" in the left thenar and was operated on by Kinoshita, who excised a part of the cutaneous tissue containing a parasite which was brought to Morishita for identification. The worm, though unfortunately partly damaged, was undoubtedly a young form of *Gnathostoma hispidum* Fedchenko. This species is a parasite generally found in the

stomach of the wild and domesticated pigs, and had never been reported before from human beings. This was, therefore, the first case of *Gnathostoma hispidum* in man.

In addition to these Ikegami (1919) reported an interesting case from Amoy, China. This case dealt also with "creeping disease" in a male Chinese, caused by a worm which was referred by him to a species of Echinorhynchus. From his description and figures the worm seems, however, to be a young form of Gnathostoma and shows a close resemblance to the specimens from the new cases described below. As the parasite in this case is, however, not determined with certainty it is not reckoned by us among the cases of human gnathostomiasis.

Out of the five above-mentioned cases four dealt with *G. spinigerum* and only one with *G. hispidum*. In man they were always found in cutaneous foci, causing abscesses, tumors, or "creeping disease." Furthermore, the specimens which occurred in man were always immature. From these facts we may conclude that the nematodes of the genus Gnathostoma cannot settle down to develop to maturity in any organ of an unsuitable host like man, but wander in the host's body, especially in the surface region, causing various cutaneous diseases. This statement applied also to the new cases which are here recorded.

PROTOCOL OF NEW CASES

1. This case was treated by Dr. Fujita of the Hankow Dojin Hospital. The patient was a male Japanese, 26 years old, who went to China in March, 1919, and the following year had "creeping disease" in the region of the right shoulder. On December 13 of that year Dr. Fujita excised the affected part and found a small nematode therein, which he afterward brought to Morishita for identification. The specimen is a small immature one mounted in balsam.

Description of the Worm.—The body is cylindrical, anteriorly truncated, posteriorly conical, with a maximum length measurement of 2 mm. by 0.29 mm. in maximum diameter which lies a little behind the middle of the body. The head bulb is provided with four rows of single posteriorly directed spines. The number of the spines in each row is 40 to 46. The spines measure about 0.01 mm. long by 5μ broad at the base. The integument has neither transverse nor longitudinal striations. The cervical sacs (*cs*) are cylindrical with a length of 0.23 to 0.3 mm. The club-shaped esophagus (*es*) measures 0.72 mm. long by 0.16 mm. wide in maximum diameter, and is surrounded by the nerve ring (*nr*) about 0.1 mm. from its anterior end. The intestine (*i*) is fairly regular and is followed by the conical rectum (*r*). The anus (*a*) lies 0.075 mm. from the caudal extremity. We have made no observation on the genital organ.

From a merely casual study since this specimen carries no body spines, it might seem reasonable to refer it to the genus Echinocephalus Molin. But Echinocephalus occurs, as a rule, in the intestine of marine fishes, has marine bivalves (*Margaritifera margaritifera, Pinna* sp.) as intermediate hosts, and has never been seen in mammals. Moreover, comparison with the specimen of Case 2, which bears body spines, though very minute, and which shows, excepting this point, a close resemblance in morphology to the specimen of Case 1, leads us to conclude that the specimen in question is referable to a species of Gnathostoma, in which the body spines have not yet developed because of immaturity. The clinical details of this case will soon be reported from Dr. Fujita's clinic.

2. This case was also treated at the Hankow Dojin Hospital by the late Dr. Kawano, who obtained a specimen from a male Japanese. The specimen is mounted in blood, now dried and partly damaged, and is labeled "cause of endemic disease." No other data are available. Dr. Fujita, who brought this specimen to Morishita, stated that the "endemic disease" may mean "creeping disease."

Description of the Worm.—The specimen is much shrunken, thick, cylindrical, 1.93 mm. long by 0.44 mm. broad in maximum diameter which lies a little behind the middle of the body. The head bulb, 0.12 mm. long by 0.21 mm. broad, bears four circular rows of spines, and is partly retracted into the so-called cuticular collar of the body. The number of the spines in each row is about forty. The spines are about 0.02 mm. long by 5μ broad at their base.

The integument of the body shows many transverse striations and annulations due to shrinkage. The anterior part for about 0.246 mm. from the cephalic end of the body proper is covered with weakly developed, uncleft, posteriorly directed spines. The esophagus is thick, club-shaped, and measures 1.04 mm. long by 0.18 mm. broad in maximum diameter. The anus is 0.055 mm. from the caudal tip. The tail projects as a small process. The genital system is still undeveloped. This specimen carries body spines. By reason of this fact the parasite is readily identified as Gnathostoma, and excepting this point is closely related morphologically to the specimen of Case 1. Both specimens may, therefore, be assumed to belong to the same species, the specimen of Case 2 being more fully developed than that of Case 1.

As to the specific name of the specimens, however, a definite decision cannot be reached, since they are so young that the structure of the body may be changed and the number of the circular rows of spines on the head may increase with the growth of the body. Notwithstanding, from the cases hitherto reported in man and their distribution, we believe that these specimens may be young forms of *Gnathostoma spinigerum.*

The nematodes of the genus Gnathostoma play an interesting rôle as human parasites. The forms found in man are always immature, and cause only cutaneous diseases, while the adult forms occur in the stomach of the host also causing pathological changes. These facts warrant the division of gnathostomiasis into two categories: gnathostomiasis externa or cutanea for the cutaneous diseases caused by immature Gnathostoma, and gnathostomiasis interna for the stomach diseases in animals caused by adult Gnathostoma.

In connection with the human cases of gnathostomiasis cutanea recorded from China it is of interest to note the frequency of gnathostomiasis in dogs, cats and hogs in China. From autopsies on eighty-five dogs from Central and North China only one case of gnathostomiasis was found, numerous gnathostomes, identified as *Gnathostoma spinigerum,* having been taken from a large stomach tumor (Figs. 7, 8). In fifty-eight examinations of cats two have been found positive for this infection. A third positive case has been found by Dr. C. McA. Wassell. Among several hundred hogs examined in Peking and in the Central Yangtze Valley no infection with *G. hispidum* has been observed. The dog and two of the cats found infected were autopsied at Wuchang, across the Yangtze River from Hankow, where the two human cases recorded in this paper apparently incurred the infection. The infection in the third cat occurred in a Peking cat autopsied by Dr. H. E. Meleney.

With reference to the route of invasion of the host it seems not unreasonable to believe that there is a free-living stage which is infective through the skin. The presence of the worms in the cutaneous tissues of man, who is only a semi-suitable host, seems to give a clue in accord with this hypothesis, since after invading the skin such worms were apparently unable to continue their course of migration through to the habitat where the adult worm is wont to develop in the appropriate host.

CONCLUSIONS

1. Five indisputable cases of human gnathostomiasis have hitherto been reported. Four of these are referable to *G. spinigerum* and one to *G. hispidum.*

2. Two new cases of human gnathostomiasis from China are added. The new cases deal with very young forms, one of which caused a typical "creeping disease." The other may have also caused the same disease.

3. Four cases of gnathostomiasis interna are recorded from dogs and cats in China. Three of these were found in cats and one in a dog. One cat was autopsied in Peking. The other infections occurred in the Hankow area.

NOTE.—The authors wish to express their thanks to Professor Seitaro Goto of the Tokyo Imperial University for his valuable suggestions and to the late Dr. H. Fujita of Hankow Dojin Hospital for kindly placing the materials at their disposal.

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Explanation of Plate XXIII

Figs. 1-3.—Gnathostoma of Case 1.

Fig. 1, Entire worm; fig. 2, anterior part of body; fig. 3, spines on head bulb, surface view.

Figs. 4-5.—Gnathostoma of Case 2.

Fig. 4, Entire worm; fig. 5, head bulb and anterior trunk, showing arrangement of spines. *a*, anus; *b*, ballonet in head; *c*, cuticular collar; *cs*, cervical sac; *es*, esophagus; *hb*, head bulb; *i*, intestine; *l*, lip; *r*, rectum.

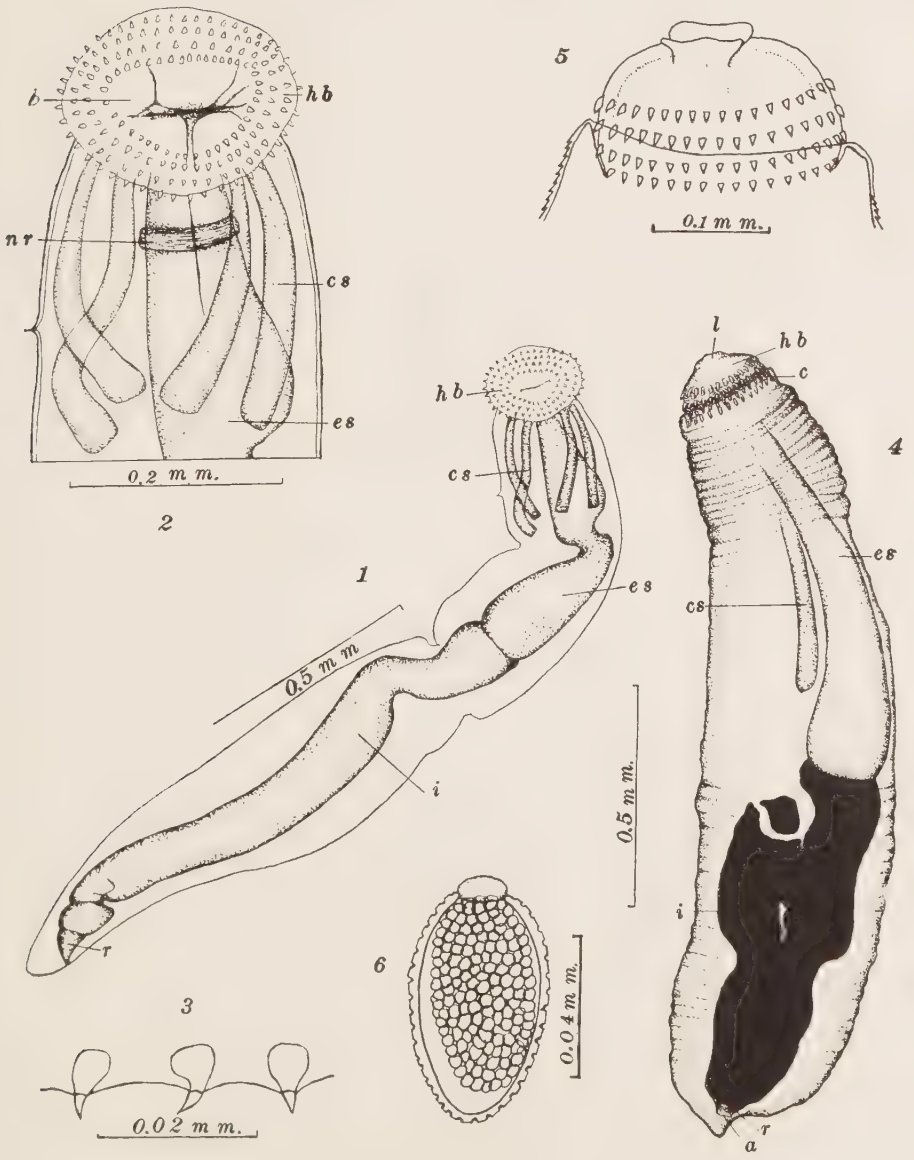
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PLATE XXIII

Explanation of Plate XXIV

Fig. 7.—Male (left) and female (right) of *Gnathostoma spinigerum* from gastric tumor of dog. × 2

Fig. 8.—Portion of stomach of dog showing tumor with two worms partly protruding from artificial opening. × 2.

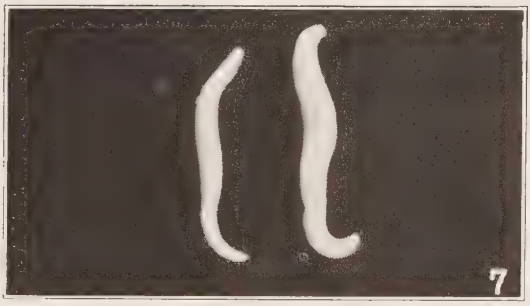

PLATE XXIV

A NEW DIECIAN CESTODE

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Several lots of cestodes collected by the late Vinal N. Edwards, at Woods Hole, Mass., from the grebes, *Colymbus hollboelli* and *C. auritus*, belong to Fuhrmann's genus of separate-sexed cestodes, Dioicocestus. While agreeing closely in anatomical details with representatives of that genus, it has not been found possible to refer them to any one of the hitherto described species. They have been referred therefore to a new species named in honor of Professor O. Fuhrmann.

Dioicestus fuhrmannii sp. nov.

Scolex: Small, about 0.4 mm. in lateral, and 0.6 mm. in dorso-ventral diameter, and about 0.4 mm. in length, provided with four small cup-shaped, muscular bothria, about 0.12 mm. in diameter; rostellum unarmed, fusiform, tapering more posteriorly than anteriorly. Although retracted in all of the scoleces seen, the musculature of the scolex and of the anterior portion of the strobile, indicates that the rostellum is protrusible, and evidently capable of considerable variation in shape (Fig. 3). The scolex itself can be more or less introverted. Strobila relatively thick, with distinct, short proglottids; entire strobila either male or female. As a rule, the female strobiles are longer, broader and thicker than the male. An average of 11 male and 11 female strobiles gave the following dimensions: males, average length, 94.3 mm., average maximum breadth, 6.6 mm.; females, average length 164.8 mm., average maximum breadth 7.4 mm. The longest male measured 164 mm. in length and 8.5 mm. in maximum breadth. The longest female was 264 mm. in length and 6.5 mm. in maximum breadth. The shortest male measured 45 mm. in length; the shortest female 108 mm. The least maximum breadth noted in a male strobile was 3.5 mm.; the least maximum breadth noted in a female strobile was 5 mm. The strobiles are from 2.5 to 3 mm. thick.

The proglottids begin about 0.5 mm. behind the scolex. In a mounted specimen, in which the proglottids began 0.5 mm. back of the scolex, there were 17 proglottids in the first millimeter of length. The proglottids throughout are short. At a distance of 20 mm. from the scolex, the length of the proglottids was 0.35 mm. The posterior proglottids were about 1 mm. in length; in a mounted specimen the posterior 15 proglottids had an aggregate length of 16 mm., an average of 1.07 mm.; the last, and longest proglottis measured 1.12 mm. in length, and the shortest of the 15, a little less than 1 mm. In the male

strobiles the genital pores are on each lateral margin, near the anterior end of the younger proglottids, but in later proglottids situated near the middle of the length. There are no genital pores in the female proglottids.

Male genitalia: The reproductive organs are duplicated in each proglottis. The cirrus is rather stout, somewhat bluntly fusiform, and is armed with strong, recurved hooks, many of them provided with long basal supports (Figs. 7, 8). In some of the largest proglottids the spines had disappeared, thus giving the deceptive appearance of smooth, unarmed cirri. The diameter of a cirrus at its largest point was about 0.16 mm., length about 0.42 mm.; length of largest hooks about 0.018 mm. The largest cirrus-pouches measured about 0.7 mm. in length and 0.4 mm. in diameter. The seminal vesicle is a relatively spacious tube, and is enclosed within the strong muscular walls of the cirrus-pouch. In some cases the cirrus pouch lies at the extreme margin of the proglottis, its lateral end even protruding from the margin. In other cases, even in the same proglottis, from one margin of which the cirrus was protruding, a cirrus-pouch, with the cirrus retracted, lies deeply embedded in the parenchyma, for the greater part of its length on the median side of the excretory vessels, and with no discernable communication with the margin. In one such case the marginal end of the cirrus-pouch was 0.8 mm. from the lateral margin of the strobila. The seminal vesicles of these embedded cirrus-pouches are full of sperm cells, while the seminal vesicles of cirrus-pouches from which the cirri are protruding, are usually empty.

In sections made from antero-median, median, and posterior regions of the strobila no testes could be found. The vas deferens could be traced as a straight, or slightly sinuous tube from the point at which it entered the median end of the cirrus-pouch, for a short distance mediad, where it lay between the excretory vessels, a little nearer to the ventral than to the dorsal vessel. In some sections, on the median side of the excretory vessels, remnants of efferent ducts could be seen which led from small spaces, usually empty, but occasionally containing a few granules. In proglottids about 7 mm. back of the scolex testes are present. They are numerous, long-oval, or fusiform, closely crowded, and form two clusters in each proglottis, which are elongated laterally, one cluster on each side of the median line. The individual testes appear to be about 60 by 18μ in the two principal diameters, the longer diameter being at right angles to the axial diameter of the strobila. In a series of transverse sections rudiments of distinct testes made their appearance in about the sixtieth section, equivalent to a distance of about 1 mm. from the scolex. It would appear that the testicular cells are soon transferred to the seminal vesicles and are emptied from the muscular ejaculatory vessel in a single copulatory act.

In transverse sections of a strobila a short distance behind the scolex the testes appeared to be in two layers, dorsal and ventral. This was at a point where the cirri were fully developed, and the seminal vesicles, enclosed in the cirrus-pouches, contained sperm cells. As many as 75 testes could be counted in a single section on each side of the median line; sections 0.015 mm. thick. About 25 mm. of the anterior end of a strobila were cut into transverse sections. Rudiments of cirrus-pouches made their appearance about 0.35 mm. back of the scolex. At this point the testes were represented by a mass of cells not aggregated into testicular bodies, which latter began to appear about 2 mm. back of the scolex. No testes were present farther back than about 16 mm. from the scolex. It would appear, therefore, that the testes develop in the extreme anterior end of the strobila, and are spent before the proglottids are pushed back as far as 20 mm., or less, from the scolex. The sperm cells are thus early transferred to the seminal vesicles, which are entirely inclosed in the cirrus-pouches, and are doubtless practically all injected from a seminal vesicle into the vagina of a female strobila at a single copulatory contact. Just before it enters the cirrus-pouch the vas deferens is enlarged to about twice the diameter it has after leaving the region of the testes, and at the same time is slightly kinked. My preparations do not show the glandular sac associated with the vas deferens, which is described by Fuhrmann in *Dioicocestus acotylus*.

Female genitalia: There is but one set of reproductive organs in the female proglottis. A genital pore is lacking, the vagina, in all cases observed stopping a short distance from the lateral margin. The lateral termination of a vagina in a mature proglottis was 0.7 mm. from the margin; in an immature proglottis it was 0.4 mm. from the margin. The seminal receptacle in this latter instance contained sperm cells. At its lateral termination the vagina enlarges slightly, and between this termination and the margin there is a tract of a peculiar tissue which is in sharp contrast with the surrounding subcuticular and muscular tissue (Fig. 10 *a*). It is composed of closely packed and very fine parallel fibers with many deeply staining nuclei, and forms a distinct path through the mesh of connective tissue, and the layers of longitudinal muscle fibers from the termination of the vagina to the lateral margin of the proglottis. Structurally this tissue bears no resemblance to the epithelium of the vagina. It evidently marks a definitely restricted path which, in the virgin proglottis, is a path of least resistance for the cirrus. There appears to be also a definite region on the cuticula which marks the lateral end of this extra-vaginal tract, and forms a restricted area at which the cirrus may penetrate the cuticula of the female proglottis. The vaginae, which are irregularly alternate, are deflected a little posteriad, in their passage toward the median line, where each is somewhat enlarged. Throughout its course the vagina is an ample tube

and functions as a seminal receptacle. Sperm cells and spermatozoa are associated together in the seminal receptacles. At its median end the seminal vesicle passes to the dorsal side of the vitelline gland, where it ends abruptly, and communicates with the germ duct by a short seminal duct. The vitelline gland is a lobed organ situated on the median line at about the middle of the length of the proglottis. One, as seen in a whole mount, measured about 0.30 mm. in length and 0.3 mm. in diameter. It lies a little toward the ventral side of the parenchymatous space. On its dorsal side is the complex of tubes shown in the diagram (Fig. 11).

The ovaries are lobed and lie symmetrically on either side of the median line, each sending a duct mediad. These unite at the median line on the dorsal side of the vitelline gland. The germ duct originates at the median line in a distinct ootype. The duct which leads from the ootype is first joined by the sperm duct, and the common duct then receives the yolk duct. Thence the duct, now the oviduct, passes anteriorly, then ventrally, as a much convoluted tube to join the uterus. Just before it enters the uterus there is a slight enlargement of the oviduct. The uterus at first is a slender tube extending laterally from the median line toward each margin, along the anterior border of the proglottis; later it gives off branches posteriorly, and ultimately comes to occupy the greater part of the parenchymatous space. The ova are small, with very thin shells, and measure from 9 to 12μ in diameter.

Musculature and structure of the strobila: There are two layers of longitudinal muscles. The character of the bundles which constitute these layers differs somewhat in different parts of the strobila, but in general their shape in transverse sections is long oval-elliptical, with the longer axis radial. These longitudinal layers are strongly developed. A weak layer of transverse fibers lies outside the outer longitudinal layer, a second lies between the two longitudinal layers, and a third and stronger layer lies within the inner longitudinal layer and surrounds the parenchymatous area. A few small bundles of longitudinal muscle fibers accompany the lateral nerve trunks (Fig. 13). These muscle fibers lie on the lateral border of the nerve, some of the fibers being more or less enclosed by the mesh of the nerve sheath. The longitudinal muscles appear as two distinct layers in series of transverse sections at above the level of the base of the retracted rostellum. The outer layer is continuous with fibers which lie immediately beneath the cuticle. So far as can be made out from my preparations the general structure of the strobila, as seen in transverse sections is as follows: Next the cuticula there is a layer of dense granular and fibrous tissue, made up of short thickish radial fibers and fine longitudinal fibers. The subcuticula is of varying thickness in different parts of the strobila, and, in deeply stained sections, appears as a mesh of radial and transverse

fibers, in which, and also in the parenchyma, were seen in a few series of sections deeply staining cells, agreeing with the "flame cells" described by Fuhrmann, as being easily seen in sections of material of *D. acotylus* which had not been subject to special treatment. Within the layer of subcuticula are the muscular layers in the order described above. The parenchymatous space merges on its exterior with the inner layer of transverse muscle. The strongest fibers of the parenchymatous area are dorso-ventral. In thickish, deeply stained sections the appearance is that of rather dense areolar tissue.

Excretory vessels: As seen in transverse sections the first vessels to appear are at the level of about the middle of the length of the scolex, where they form several short irregular coils laterally placed with respect to the rostellum. These coils soon become connected by communicating branches which pass dorsal and ventral to the rostellum and form a plexus of vessels which continues for a short distance into the strobila, but not so far as to the base of the retracted rostellum. Near the base of the rostellum the excretory vessels are represented by two vessels toward each margin, which continue as dorsal and ventral vessels throughout the length of the strobila. They are sinuous, in close spirals, the ventral being much larger than the dorsal. Near the posterior end of each proglottis the ventral vessels are connected with each other by a relatively large transverse vessel. The walls of the ventral vessels are relatively thin, with few cells, while the walls of the dorsal vessels are relatively thick, with many cells (Fig. 12). No dorsal connecting vessel was observed.

Nervous system: So far as shown in sections the structure of the nervous system is in close agreement with that described by Fuhrmann for *D. acotylus*. The lateral nerve trunks traced anteriorly end in ganglia which lie on either side of the retracted rostellum, and are connected with each other both dorsally and ventrally. The lateral nerve trunks are accompanied throughout their course by two smaller nerve trunks, one dorsal and the other ventral, on the lateral side of the main nerve trunk (Fig. 13). The nerve trunks are surrounded by relatively thick connective tissue sheaths, and are accompanied by a few longitudinal muscle fibers. The lateral nerve trunks in anterior portions of the strobila are small but are accompanied by the two small by-nerves. The latter were noted in sections very near the ganglionic masses.

It is noted as a characteristic of the different species of this remarkable genus of cestodes hitherto described that a male and a female strobila are always to be found together. The same characteristic is indicated by most of the material collected by Mr. Edwards. There are two dates on which a single specimen is recorded, and one date on which apparently an odd number were found. In the absence of definite notes made at the time of collecting, these records should be regarded as

incomplete. On April 28, 1913, one strobila, female, was taken from *Colymbus auritus*. Sections made from the posterior end of this strobila showed that the seminal receptacles were full of sperm cells, thus indicating that at least one male strobila had been present in this host. This association of paired strobiles makes it especially desirable to know the life history, and more particularly the early stages of development of these monosexual strobiles.

The species of the genus Dioicocestus recorded by Fuhrmann are:

D. acotylus from *Colymbus dominicus.*

D. aspera from *C. griseigena,* and *C. cristatus.*

D. novae-hollandiae from *Colymbus* sp.

D. paronai from *Plegadis guarauna.*

There is rather close agreement in the anatomy of the strobiles of the several species of the genus Dioicocephalus which have been described, and the species represented by Mr. Edwards' collection agrees well in many particulars with them, especially with *D. acotylus* and *D. aspera,* but the presence of distinct bothria precludes the former, and the absence of spines from the rostellum precludes the latter species.

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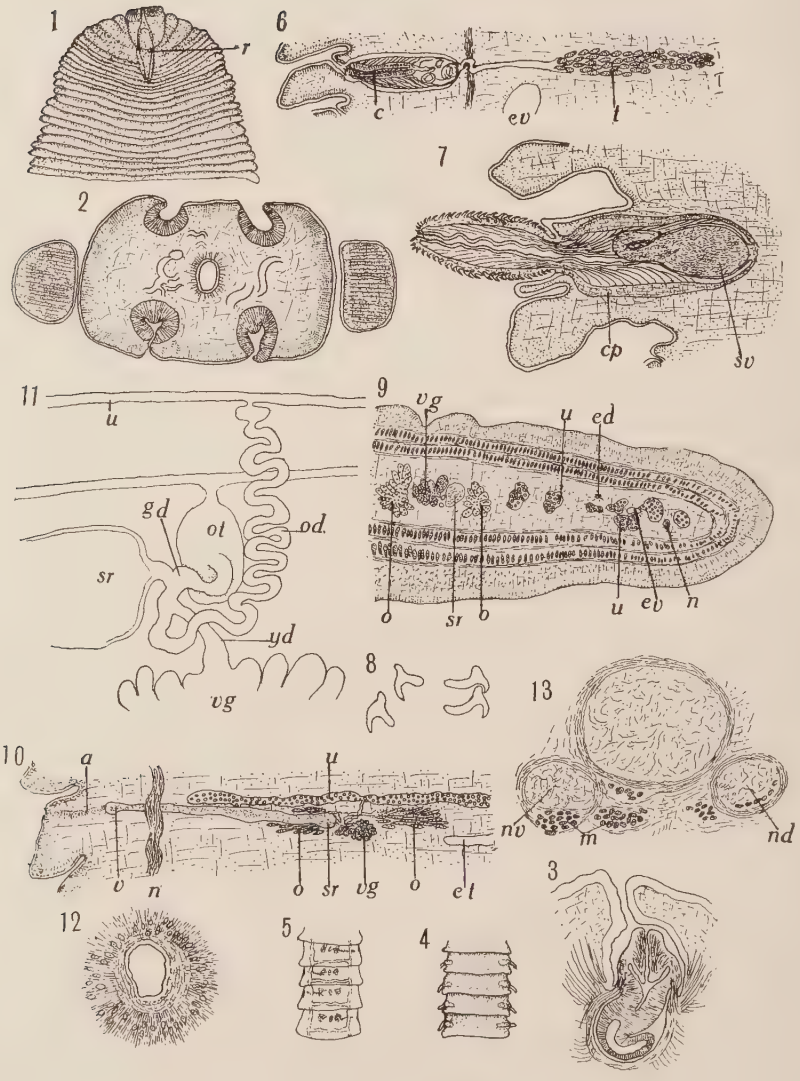
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PLATE XXV

Explanation of Plate XXV.

Abbreviations

c cirrus	*ot* ootype
cp cirrus-pouch	*r* rostellum
ed dorsal excretory vessel	*sr* seminal receptacle
ev ventral excretory vessel	*sv* seminal vesicle
gd germ duct	*t* testes
n nerve	*u* uterus
nd dorsal by-nerve	*v* vagina
nv ventral by-nerve	*vg* vitellaria
o ovary	*yd* vitelline duct
od oviduct	

PLATE

Fig. 1.—Anterior end of strobila, balsam; diameter of scolex, 0.16 mm.

Fig. 2.—Fourth section in a series of transverse sections, thickness about 0.16 mm.; diameters of section of scolex 0.42 by 0.63 mm. The four bothria, sheath of rostellum and plexus of excretory vessels are shown, also portion of the strobila on each side of the scolex.

Fig. 3.—Portion of frontal section of scolex, showing rostellum and sheath; length of rostellum about 0.43 mm.

Fig. 4.—Posterior proglottids of male strobila; breadth 4 mm.

Fig. 5.—Proglottids from middle of female strobila; breadth 4 mm.

Fig. 6.—Frontal section of male proglottis; length of cirrus-pouch 0.48 mm.

Fig. 7.—Section of cirrus and cirrus-pouch, with seminal vesicle filled with spermatozoa and sperm cells, length of cirrus-pouch 0.48 mm.

Fig 8.—Hooks from cirrus; length 18 mm. The hooks with the elongated basal supports are from the middle of the cirrus.

Fig. 9.—Portion of transverse section of female proglottis; lesser diameter of section about 1.10 mm.

Fig. 10.—Frontal section of female proglottis; *a*, tract of special tissue which lies between the vagina and the margin of the strobila; length of proglottis 0.7 mm.

Fig. 11.—Diagram of female genital ducts; constructed from about ten consecutive sections of 0.01 mm. thickness, in same series as that from which Figure 10 was made.

Fig. 12.—Transverse section of dorsal excretory vessel; longer diameter of lumen about 0.03 mm.

Fig. 13.—Transverse section of a nerve trunk; *m*, longitudinal muscle fibers; diameter of main nerve about 0.09 mm.

A STUDY OF TRICHOMONAS FROM THE GUINEA-PIG *

MISAO TANABE

In 1914 and 1918, Kuczynski published papers in which he described in detail the cell structure and division process of the Trichomonas from the guinea-pig. In 1921, Faust found a Trichomonas in guinea-pigs in Peking, which he considered different from that described by Kuczynski and gave it the name *Trichomonas flagelliphora* Faust, 1921. In 1921, Wenrich published a paper on the trichomonas from mice in which he mentioned Trichomonas from guinea-pigs. With the intention of studying comparatively as many species as possible, I first began with Trichomonas from guinea-pigs and since I have found the structure to differ somewhat from that described by the authors mentioned above, I think it would be worth while to record my findings on Trichomonas from guinea-pigs in Baltimore.

The material on which this study is based was obtained from laboratory guinea-pigs. The total number of guinea-pigs examined was five, all of which were infected with Trichonomonas but to different degrees. I was able to find dividing forms of Trichomonas among the parasites from every animal, but two animals were more favorable for the study of the division process, so the study of division phenomena was carried out on the parasites from these two animals. Smears were always made on cover glasses from the contents of the cecum as soon as the animal was killed and fixed while wet. As fixatives, three kinds were used in each case; namely, Schaudinn's fluid with glacial acetic acid, 1 per cent. chromic acid solution and Flemming's solution (strong solution without glacial acetic acid, diluted 2-3 times with water). The latter two were used for the special purpose of properly fixing the parabasal body. As stains, Heidenhain's iron hematoxylin method only was used; leaving slides in 4 per cent. iron alum solution from 6 hours to overnight and then in 0 5 per cent. hematoxylin from 4 hours to overnight and destaining in 2 per cent. iron alum solution.

VEGETATIVE FORM

According to Kuczynski, the size of Trichomonas from the guinea-pig is 15 to 22μ in length and 10 to 15μ in width. Recently Faust found a small Trichomonas from guinea-pigs in Peking that is 8 to 14μ long and 6.5 to 10μ wide. In my case, the measurement of the parasites was

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carried out with the aid of the ocular micrometer on the slides fixed with Schaudinn's fluid. Only typical parasites were measured, i. e., those with the body fusiform in shape and axostyle straight. As a result, four guinea-pigs were found to harbor parasites very much alike in size (11 to 22μ in length and 7 to 14μ in width), but the fifth animal contained a smaller form (8 to 14μ in length and 5 to 8μ in width). In this animal I tried to find larger specimens than this, but did not succeed. The large Trichomonas from four of the guinea-pigs seem to be the same kind as those described by Kuczynski and the smaller specimens from the fifth animal resemble those reported by Faust. From the results of my measurements, there appear to exist two forms of Trichomonas in guinea-pigs having the same structure.

STRUCTURE

Faust's description of the structure of Trichomonas from guinea-pigs varies in several points from that of Kuczynski. My findings differ to some extent from those of either of the above mentioned authors. No differences in structure were noted in the trichomonads from the different hosts examined by me.

The parasite is fusiform in shape; the anterior end being blunt, the posterior end tapering slightly and the dorsal side appearing more convex than the ventral. In the protoplasm, especially at the posterior half of the body, vacuoles and granules are scattered about, sometimes abundantly; stained deeply with hematoxylin (Fig. 2). The nucleus is oval or ellipsoidal in shape, being about 5μ in length and about 3μ in width, and lies near the anterior end of the body and to the left of the middle line. It is of the vesicular type with a very thin nuclear membrane, inside of which several tiny chromatin granules can be seen, and a small round karyosome which is located sometimes in the center and sometimes near one side (Fig. 1). The nuclear membrane appears distinctly in material fixed with Flemming's solution and 1 per cent. chromic acid solution, but this was not the case in material fixed with Schaudinn's fluid (Figs.1, 2). Around the karyosome there usually can be seen a narrow faint halo in specimens suitably stained. The inside of the nucleus is composed of a network of small chromatin granules. In stained slides from four animals all parasites except dividing forms exhibit the nuclear structure above described, but in one animal, No. 4, many parasites show various structures similar to the figures of Kuczynski (see his Taf. 11, Figs. 1-8). Whether these specimens were undergoing changes, or whether there is some other cause could not be ascertained, but I hope to clear up this point by further study. At the anterior end of the body there exists a small granule called the blepharoplast, from which arise three anterior and one posterior flagella and the undulating membrane. I cannot state

definitely whether the blepharoplast consists of one or two granules; but there was no evidence of a rhizoplast, connecting the blepharoplast and the nucleus. The anterior flagella are three in number, ending freely immediately after arising and they appear approximately alike in every respect, averaging about one half the length of the body. Along the dorsal side of the body is the undulating membrane, making 5 to 7 undulations; and along the outer edge of this membrane a heavy flagellum runs backward, arising from the blepharoplast, and ending as the free flagellum of about the same length of the anterior flagella.

The chromatic basal rod arises from the blepharoplast; usually presents a slight buckle shortly after arising; lies along the dorsal edge of the body, and ends near the axostyle. It is thickest at about the middle of its length and tapers towards both ends. Along the inner side of the chromatic basal rod, but not attached to it, is a row of chromatic granules which seem to extend from the blepharoplast to the posterior third of the rod; this is called the outer row of chromatic granules by Wenrich in the Trichomonas from mice.

The axostyle is situated along the longitudinal axis of the body and ends in a point, projecting to various lengths from the posterior end. At the point of emergence of the axostyle from the body, there is always a ring of chromatin granules. In this Trichomonas, I could not make out the anterior end of the axostyle, on account of its faint reaction to the stain and of the presence of a group of chromatin granules anterior to the nucleus which sometimes were present at the anterior tip of the axostyle, but sometimes not (Figs. 1, 2). Wenrich in the Trichomonas from mice noted an inner row of chromatin granules, which were arranged along the dorsal side of the axostyle. In the Trichomonas from guinea-pigs, I also found this chromatic row, although not so distinct as that in the Trichomonas from mice, but strange to say, it appears very distinctly in the later stages of division (Fig. 16). Posterior to the nucleus, there is always found a group of chromatin granules, which sometimes look as if arranged in 4 to 5 rows, and anterior to the nucleus, there can also be seen a group of chromatin granules, as mentioned above, very few in number, but these two groups of granules cannot be seen in the late stages in the division of the parasite. Near to the blepharoplast on the ventral side of the body is a cytostome.

The presence of the parabasal body in Trichomonas was first described in Trichomonas from frogs by Janicki. This body is peculiar since it is dissolved in sublimate or acetic acid, and therefore fixatives containing these two are not suitable for bringing out this structure (Janicki, Cutler and Wenrich). Kuczynski found the parabasal body in Trichomonas from guinea-pigs, but in very few cases. Wenrich simply states that he could find the body in the Trichomonas from

guinea-pigs. According to Wenrich, a great majority of the Trichomonas from mice showed this body when fixed with weak Flemming's solution. In material from another animal, however, fixed with 1 per cent. chromic acid solution and Flemming's solution without glacial acetic acid, only a small percentage of the parasites exhibited the body. In my study of Trichomonas from guinea-pigs, I have had the same experience as that of Wenrich in regard to the parabasal body in the parasites fixed with Flemming's solution without glacial acetic acid and with chromic acid solution. Besides this, however, I have had a similar experience with material fixed with Schaudinn's fluid; that is, a large number of the flagellates from animal No. 1 showed this body while it was exhibited in very few flagellates from No. 4. Generally, in material fixed with Flemming's solution or chromic acid solution, the parabasal body appears a little fainter than the chromatic basal rod, but this is not the case in material fixed with Schaudinn's fluid; that is, in the latter it stains as deeply as the chromatic basal rod. Moreover, the parabasal body in material fixed with Schaudinn's fluid seems in general to be a little narrower and shorter (Fig. 1) than in material fixed with Flemming's or chromic acid solution (Figs. 2, 15, 18).

This parabasal body seems to arise near the blepharoplast, but a connection between the two has never been found and a very narrow space always separates them. It is cylindrical, of various lengths and shapes and usually lies between the outer chromatic row and the nucleus; and ends after increasing in diameter near the distal end of this chromatic row (Figs. 2, 15, 18). It appears distinctly in material fixed with Flemming's and chromic acid solutions. I have also found the parabasal body in Trichomonas from some species of termites (fixed in Flemming's solution without acetic acid and in chromic acid solution) but never in material fixed with Schaudinn's fluid. The fact that there appear to be individual variations in the parabasal body in parasites from different animals makes it a very interesting structure to study.

On some of my slides I have found a peculiar form such as is shown in figure 3 which is similar to specimens from mice described by Wenrich. I cannot account for this peculiar form, although it may be a degenerating parasite, as maintained by Wenrich. Besides this, I have found forms such as is shown in figure 17. This is considered the cyst of Trichomonas by some investigators but its significance is not yet clear.

THE DIVISION PROCESS

Kofoid and Swezy have described in several species of Trichomonas multiple as well as binary fission. Contrary to this, Kuczynski could not find multiple fission in any of four species of Trichomonas—*T. augusta, T. muris, T. caviae* and *T. eberthi*—and Wenrich also was

unable to find this type of division in *Trichomonas muris*. During my study, I have found a large number of parasites undergoing binary fission, but never any in the process of multiple fission.

The initiation of division can be indicated first by changes in the nucleus. The scattered chromatin granules begin to form chromosomes; the numerous small granules begin to gather into large masses (Fig. 4) and ultimately produce six chromatin masses, the prophase chromosomes (Fig. 5-8). There is no definite agreement among investigators with regard to the number of prophase chromosomes in any Trichomonas. In the Trichomonas from the guinea-pig, Kuczynski counted 8 chromosomes, whereas Wenrich states in his paper on *Trichomonas muris* that, "As for other species, since Kuczynski finds and figures conditions in *Trichomonas caviae* so similar to those in *Trichomonas muris,* I am inclined to believe that there are 6 chromosomes in *T. caviae.*"

It is very difficult to determine the number of chromosomes because of the inequality in their size and the presence of some chromatin granules near the nuclear membrane. Besides this some chromosomes have already divided into two while others are still undivided (Fig. 6). I could not decide the number of chromosomes until I found the forms shown in figures 8, 10, 12. I have encountered others similar to these, so that there is little doubt but that the number of the chromosomes in the Trichomonas from the guinea-pigs is 6. During division the 6 prophase chromosomes gather in the middle of the nucleus, and each finally divides longitudinally into two. The metaphase chromosomes are also 6 in number (Figs. 10, 12). Coincidently with the first changes in the nucleus, a new chromatic basal rod begins to grow out from the blepharoplast (Fig. 4). The blepharoplast divides and one daughter blepharoplast migrates to the other end of the nucleus, exhibiting a distinct paradesmose (Fig. 8). The two groups of 6 chromosomes then draw near the blepharoplasts. Then the nucleus begins to elongate, constricts in the middle, and finally separates into two. During the process of nuclear division, the nuclear membrane remains intact.

The new chromatic basal rod, which grows out from the blepharoplast, is situated dorsal to the nucleus in the beginning (Fig. 4), but changes its position with the appearance of the paradesmose (Fig. 8). Whether the new chromatic basal rod arises from the original blepharoplast or from one of its daughters could not be determined because the structure of the blepharoplast is very difficult to make out. Shortly after the outgrowth of the new chromatic basal rod, there begins to appear the flagellum that will run along the margin of the chromatic margin along the new undulating membrane (Fig. 9). Judging from figures 8 to 16, this flagellum appears to arise from the new blepharoplast, and does not result from the splitting of the old flagellum. It is very difficult to follow the division process of the anterior flagella,

but it seems that one or two parental flagella pass to the one daughter parasite and the new ones appear to complete the number in each daughter parasite. The outer row of chromatin granules is observed during the whole process of division, and I could not find any changes at any stages of division. The inner row, although recognizable during the early division process, appears very distinctly in the latter stage of division. As for the division of the axostyle of Trichomonas, there are two opinions among investigators. According to one, during the division of the parasite, the old axostyle divides whereas according to the other, the old axostyle disappears during division, and a new one grows out from each blepharoplast. In the Trichomonas from the guinea-pig, Kuczynski maintains that two new axostyles grow out after the disappearance of the old one. I tried to decide this point in the Trichomonas from the guinea-pig, but was not successful because the axostyle stains very poorly and it is often difficult to trace it throughout its whole length. Judging from figures 8 to 16, however, an axostyle seems to grow out from each new blepharoplast after the disappearance of the old one.

Tracing the division of the parabasal body is very difficult, and no one has described it definitely. In my slides, all dividing forms with one exception, have shown two separate newly produced parabasal bodies (Fig. 15). I have, however, found one case, shown in figure 18, in which the parabasal body seems to be dividing. I think this is a real division of the parabasal body because of the following points: (1) No one has yet described a Trichomonas which has two parabasal bodies. (2) I have found the same appearance in Trichomonas from termites in which other organelles are in division. The division of the cytoplasm begins after all the organelles are duplicated. It seems to complete the process very rapidly, as mentioned by Dobell in the trichomonas from frogs, because I could not find any dividing specimens in which the two daughter parasites are connected with only a thin filament of cytoplasm, although such forms as are shown in figure 16 were present in large numbers.

SUMMARY

The Trichomonas from the guinea-pig in Baltimore agrees with that described by Kuczynski, except that

1. From the standpoint of size; one large and another smaller species seem to exist.

2. An inner row of chromatic granules is present, although not so distinct as in Trichomonas from the mouse.

3. Anterior to the nucleus, there is a small number of chromatic granules. Posterior to the nucleus, there is usually a group of chromatin granules which often seems as if arranged in 4 or 5 rows.

4. There is a chromatic ring where the axostyle emerges from the posterior end of the body.

5. The parabasal body of the parasite seems to vary in nature, in parasites from different animals, as shown by different methods of fixing and staining material.

6. In both prophase and metaphase, there are always found 6 chromosomes.

Many thanks are due Drs. R. W. Hegner and L. R. Cleveland for valuable suggestions and help.

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Explanation of Plate XXVI

All figures, unless otherwise indicated, are camera lucida drawings of specimens fixed with Schaudinn's fluid and stained in iron alum hematoxylin. Magnification × 1925.

Fig. 1.—Typical vegetative form.

Fig. 2.—Typical vegetative form, fixed with Flemming's solution without acetic acid. Nuclear membrane is more distinct than that of figure 1 and parabasal body stains fainter but is longer and wider.

Fig. 3.—Vegetative form with much enlarged nucleus full of large granules stained deeply with hematoxylin.

Fig. 4.—Early division stage with the first changes in the nucleus and the outgrowth of a new chromatic basal rod.

Figs. 5 to 7.—Division forms with 6 double chromosomes and new chromatic basal rods.

Fig. 8.—Division form with 6 double chromosomes. In this individual the two blepharoplasts have already separated, showing a distinct paradesmose.

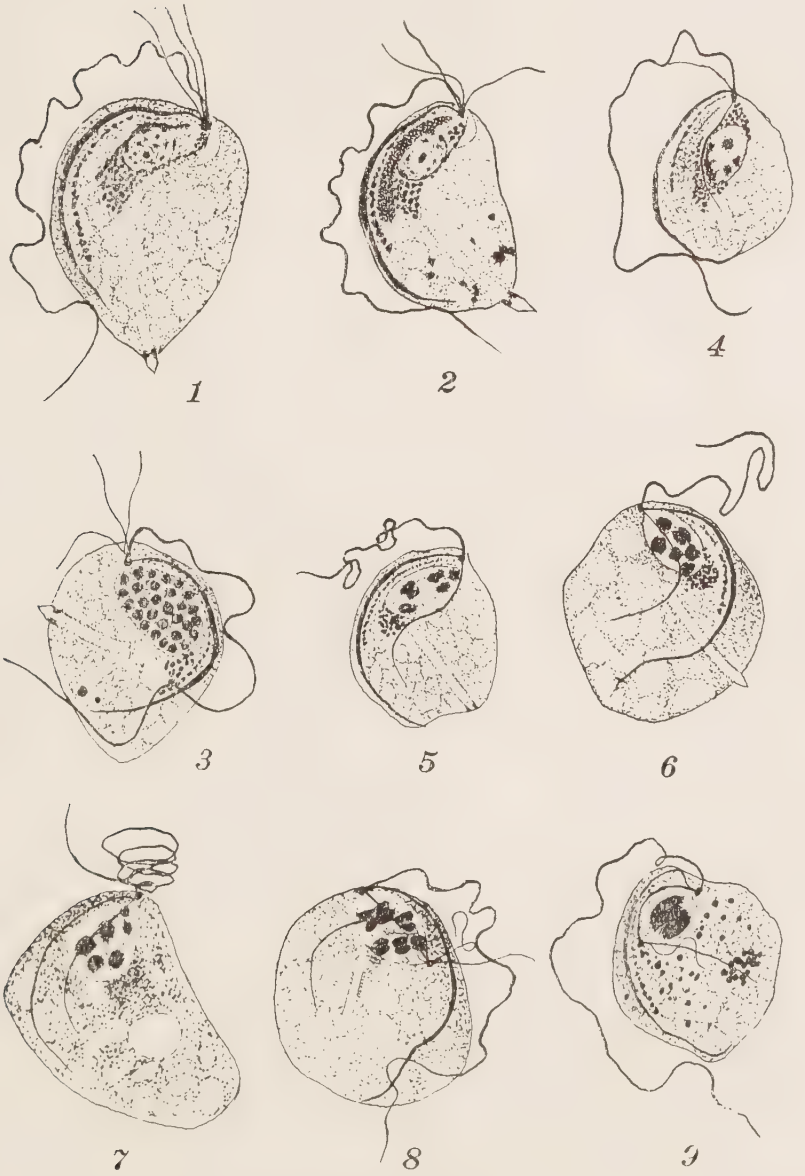


PLATE XXVI

Explanation of Plate XXVII

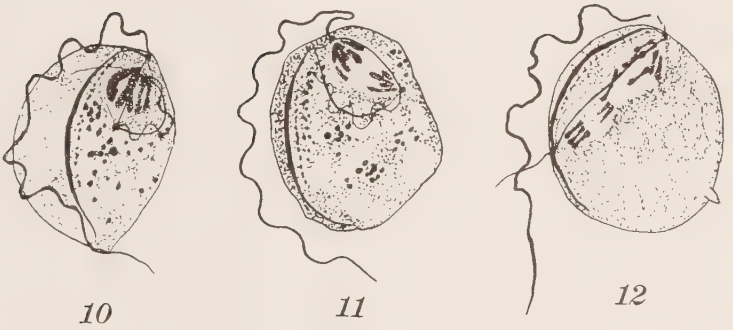
Figs. 9 and 10.—Division forms, with division spindles. In figure 9, the flagellum of the new undulating membrane is appearing. The structure of the spindle is not clear. In figure 10 the number of chromosomes is 5, one of which is twice as large as the others.

Figs. 11 and 12.—Chromosomes are dividing in anaphase. In figure 12 the nucleus is elongated and slightly constricted in the center.

Figs. 13 to 16.—Telophase. In figures 13 and 16, chromosomes can be seen distinctly. In figure 15, fixed with Flemming's solution, each new nucleus has one parabasal body. In figure 16, two axostyles and inner rows of chromatin granules are distinctly visible.

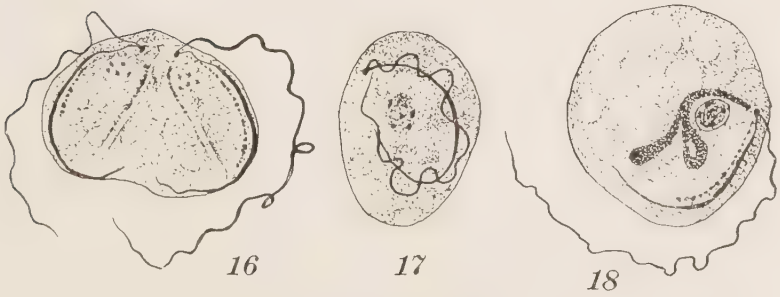
Fig. 17.—Supposed cyst stage.

Fig. 18.—Stage exhibiting what appears to be a dividing parabasal body. Fixed with Flemming's solution.



10 11 12

13 14 15



16 17 18

PLATE XXVII

SOCIETY PROCEEDINGS

THE FORMATION OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

For several years the feeling has grown that an organization is needed to represent more adequately the large group in the United States working in the field of parasitology. There are few subjects which command the interest of workers in such widely different fields. The interest of zoologists in parasitology has increased greatly in the last few years so that the number of institutions which have parasitologists on the staff of the department of zoology and give courses in this subject, is already large and constantly increasing. Veterinary parasitology in the United States dates back several decades and represents a very important and constantly expanding field. A related phase of the problem, the study of the parasites of fishes and other food animals, is already receiving considerable attention and promises well for the future. The remarkable advances which have been made in the last two or three decades in our knowledge of the animal parasites of man and in the insect transmission of disease is reflected in the United States in the increased interest of medical and public health men in the subject of parasitology. The acquisition of tropical colonies by the United States, the building of the Panama Canal, the increased commerce with tropical America and other tropical regions and the realization of the importance of parasitic diseases in the United States itself has developed an interest in the investigation and control of the human diseases produced by animal parasites or transmitted by insect vectors, which can hardly be realized by one who has not followed the development in this field. A definite demand has, accordingly, arisen for an organization which will bring together zoologists, veterinarians and medical and public health men who have a common interest in developing the important subject of parasitology.

Last spring letters were sent out from a group of parasitologists in Washington to a number of persons interested in the subject, inquiring their opinions in regard to the formation of a society of parasitologists. The great majority of the answers received were enthusiastically in favor of such an organization. It was therefore decided to call a meeting of parasitologists at the Washington meetings of the American Association for the Advancement of Science in order to discuss the formation of an American society for parasitologists. In preparation for this meeting, the parasitologists of Washington and Baltimore appointed a

committee consisting of R. W. Hegner, chairman; Eloise B. Cram, secretary; B. H. Ransom and W. W. Cort. This committee was asked to call a meeting of the parasitologists, to prepare a tentative Constitution and By-Laws for the proposed society and to make nominations for the first set of officers. The meeting was called for 4:30 p. m., Tuesday, December 30 at the Central High School in Washington and was attended by thirty-two persons representing all phases of parasitology and practically all sections of the country.

MINUTES OF MEETING HELD IN CENTRAL HIGH SCHOOL

The group was called to order by W. W. Cort as president of the Helminthological Society of Washington. G. R. LaRue was elected chairman for the meeting and W. W. Cort, secretary.

The report of the committee appointed by the parasitologists of Washington and Baltimore to consider the formation of a society of parasitologists was presented by R. W. Hegner, chairman. The committee recommended (1) that a society be organized to be called the American Society of Parasitologists; (2) that a Constitution and By-Laws for the first year, 1925, be adopted as follows:

CONSTITUTION AND BY-LAWS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

Name and Object

The name of the society shall be the American Society of Parasitologists.

The object of the Society shall be the association of workers in the field of Parasitology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of parasitological teaching and investigation in this country.

Membership

The members of the Society shall be of two classes, active and foreign honorary.

Any person interested in parasitology shall be eligible for active membership.

Any foreign scientist who has made eminent contribution to parasitology shall be eligible for honorary membership.

Candidates for membership shall be elected by ballot at the annual meeting upon recommendation by the Council.

Officers

The officers of the Society shall be a President, a Vice-President, a Secretary and a Treasurer, who shall be elected by ballot for one year, and members at large of the Council.

The Council shall consist of the President, the Vice-President, the Secretary, the Treasurer and eight members elected by ballot from the Society at large, two for four years, two for three years, two for two years and two for one year. After the first year, two members at large of the Council shall be elected each year to serve four years. If any member at large shall be elected to any other office in the Society, a member at large shall be elected immediately to serve out the remainder of his term.

The affairs of the Society shall be managed by the Council.

Five shall constitute a quorum of the Council.

DUES

The dues shall be one dollar per year unless changed by vote of the Society at an annual meeting.

MEETINGS

There shall be an annual meeting and such other scientific or business meetings as the Council shall determine.

The annual meeting shall be known as the business meeting when the election of officers for the ensuing year shall be held and routine and other business transacted.

This constitution and by-laws shall be in force for one year and until a substitute shall be adopted. At the annual meeting in 1925 a permanent consitution and by-laws shall be submitted by the Council for the consideration of the Society.

The following officers were nominated for the Society:

President.....Henry B. Ward
Vice President.....S. T. Darling
Secretary and Treasurer.....W. W. Cort

MEMBERS AT LARGE OF THE COUNCIL

For One Year	*For Two Years*
F. C. Bishopp	W. A. Riley
C. A. Kofoid	E. E. Tyzzer
For Three Years	*For Four Years*
R. W. Hegner	P. S. Bartsch
B. H. Ransom	C. W. Stiles

On motion, the report of the committee was accepted as a whole, and the secretary was instructed to cast a unanimous ballot for the officers as nominated.

It was then voted that the following paragraph be added to the by-laws:

The charter membership shall consist of those persons nominated as officers and members of the Council together with such others as shall during the years 1924 and 1925 pay the dues and be duly approved by the Council.

It was voted that the Council be empowered to fill any vacancies in the officers or Council which come about through the inability or refusal of those elected at this meeting to serve.

On motion it was voted that the Society apply for affiliation with the American Association for the Advancement of Science and the Council was instructed to take up at an early date the question of affiliation with other societies.

The enthusiasm and unanimity of those present augers well for the future of the new society.

The first activity of the society will of necessity be the building up of its membership. It will be noted from the constitution that anyone

interested in parasitology is eligible for membership. The secretary-treasurer will be pleased to receive applications for membership, accompanied by one dollar for dues for the first year.

Future activities of the society will be developed by the council on the advice of the membership. One of the most important activities will undoubtedly be the arrangement of meetings for the presentation of papers in parasitology. The council will welcome suggestions from any one interested in regard to the policies or activities of the new society.

W. W. Cort, Secretary-Treasurer,
American Society of Parasitologists.
Address at School of Hygiene and Public Health,
Johns Hopkins University, Baltimore, Md.

BOOK REVIEWS

ANKYLOSTOMIASIS AND BILHARZIASIS IN EGYPT. Reports and Notes of the Public Health Labs., No. 6, Cairo, Egypt, 1924, 196 pp. Compiled by M. KHALIL.

This progress report of the Ankylostoma and Bilharzia Consultative Committee covers ten years of work. The first part gives a history of the extensive control measures instituted against ankylostomiasis and bilharziasis in Egypt and shows very definite progress made in combating these two diseases. There is also included an exhaustive bibliography on these two diseases in Egypt. From experience with carbon tetratchloride in *Ancylostoma duodenale* infection the conclusion is drawn that this drug is a valuable and safe anthelmintic for this hookworm and is more effective than either oil of chenopodium or thymol; the adult dose of 5 cc. advocated is rather larger than used by most workers. Part three contains an interesting analysis of methods of control of bilharziasis in Egypt, wtih extensive experiments on the use of copper sulphate in eradicating snails. The attempt to exterminate the snail intermediate hosts is considered to be the most promising control measure against this disease in Egypt. It is suggested that as measures to kill the snails the summer rotation of irrigation in Egypt be utilized in drying up the small irrigation canals, that the small amount of water left in these canals be treated with copper sulphate and that the intakes of the canals be guarded against the introduction of snails.

Parts four and five bring out very clearly the high incidence of parasitic infestations in Egypt and the great importance to the health of the population of the diseases which they produce. Part six is a description of a new trematode of the rat, *Echinostoma egyptica.* Part seven a study of the complement fixation reaction in bilharziasis by the use of *Fasciola hepatica* extract as an antigen, and part eight a study of the classification of the family Ancylostomidae. It is hard to understand just why these three papers were included since they have little if any connection with the main subjects dealt with.

The report as a whole gives the impression that the Ankylostomiasis and Bilharziasis Consultative Committee is going about its problem in a systematic manner and is trying to build up an adequate scientific basis for the control work which it is undertaking.

TRANSACTIONS OF THE FIFTH BIENNIAL CONGRESS OF THE FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE, SINGAPORE, 1923. Edited by The Hon. DR. A. L. HOOPS and DR. J. W. SCHARFF. London: John Bale, Sons and Danielsson, Ltd., 1924, 995 pp., 86 figs.

The impressive volume which records the transactions of the Singapore Congress of the Far Eastern Association of Tropical Medicine demands more than mere passing attention. Evidently the work of such a congress would be deeply concerned with problems of parasitology and equally clearly would devote attention to the clinical side of matters to such a degree as to obscure important findings in the field of parasitology. It would be impracticable with the space available to review adequately the work of the congress as presented in this splendid volume of 995 pages but it is full of material that is of great interest to the parasitologist. Even in the strictly clinical sections of the work one finds a multitude of minor items that are of such importance as to well repay the parasitologist for its detailed study. It will be impossible here even to refer to many of these important features. Certain items have been selected in full recognition of the fact that such mention does not do justice to the work as a whole.

The section on malaria includes significant studies on the Anopheline mosquitoes, on their habits and control, as well as on epidemics and clinical features of malaria, especially in its relation to mental diseases. Other significant sections deal with hookworm disease and its treatment by carbon tetrachloride and with the plague. In the former special mention should be made of the careful study by Oudenal on the pathology of the intestinal wall in cases of ankylostomiasis. In the latter special mention should be made of papers on the original center of this disease and its occurrence in wild rodents.

Among the many other important papers, special mention might be given to the survey of recent progress in parasitology made by Japenese investigators. In this paper Professor Miyagawa has brought together in clear and concise fashion a survey of the unfortunately widely scattered but epoch-making recent work on helminthes by a long series of distinguished Japanese investigators. To those who have experienced the difficulties encountered by many parasitologists in securing accurate and complete information concerning these researches, this paper will be of great value. It is unfortunate that it might not have been accompanied by more extensive citations which would be desirable in securing information in greater detail concerning any particular item.

From the parasitology laboratory of Peking Union Medical College is printed a comparative study of *Clonorchis sinensis* by Ch'en Pang. The author has made an intensive and thorough study of material collected from man and several other hosts and obtained in a series of widely separated localities in China and Korea. In connection with the work of Kobayashi in 1915 on the same parasite in Japan it supports what may well be a final conclusion that although specimens differ widely in size, all of these forms must be assigned to the single species rather than to two types with separate names, as originally proposed by Baelz and reaffirmed by Looss.

To the parasitologist and especially to the modern doctor who regards Ascaris as a casual parasite of passing interest should be commended a careful perusal of the paper on surgical Ascaridiosis by Le Roy de Barres with its overwhelming mass of evidence well organized to show the dangers associated with the parasitism of this species.

The work is well printed and splendidly illustrated for which the publishers deserve special commendation. Undoubtedly much of its value is attributable to the work done by the editors to whom also congratulations should be extended. One may fondly hope that subsequent congresses may be equally well served for a succession of such volumes would form a most important part of the literature of parasitology.

NOTES

The series of Collected Papers of the Department of Helminthology at the London School of Tropical Medicine has been extended by the publication of Part V. The items included therein deal with many points from the eelworm disease of potatoes to the present day teachings on helminthology in relation to public health. They demonstrate the breadth and value of the work being done in this field at that institution.

Experimental Studies on Yellow Fever in Northern Brazil by Noguchi and his associates form Monograph 20 of the Rockefeller Institute for Medical Research. Important data were secured concerning strains of *Leptospira icteroides* and its pathogenicity for monkeys, guinea-pigs and young dogs.

The Institute for Medical Research Kuala Lumpur Federated Malay States has recently published Studies No. 18 on the Treatment of Malaria with the Alkaloids of Cinchona.